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(54) Treatment of osteoporosis with EP2/EP4 receptor selective agonists

(57) This invention is directed to methods and pharmaceutical compositions comprising prostaglandin agonists which are useful to prevent bone loss, restore or augment bone mass and to enhance bone healing including the treatment of conditions which present with low bone mass, such as osteoporosis, and/or bone defects in vertebrates, and particularly mammals, including humans. This invention specifically relates to methods and pharmaceutical compositions comprising combinations of EP<sub>2</sub> receptor selective agonist and EP<sub>4</sub> receptor selective agonists and to methods and pharmaceutical compositions comprising agents which are agonists for both the EP<sub>2</sub> receptor and the EP<sub>4</sub> receptor.



(11) EP 1 121 939 A2

EP 1 121 939 A2

Description

BACKGROUND OF INVENTION

[0001] This invention relates to methods and pharmaceutical compositions comprising prostaglandin agonists which are useful to prevent bone loss, restore or augment bone mass and enhance bone healing including the treatment of conditions which present with low bone mass and/or bone defects in vertebrates, and particularly mammals, including humans. This invention specifically relates to methods and pharmaceutical compositions comprising EP<sub>2</sub> receptor selective agonists and EP<sub>4</sub> receptor selective agonists and to methods and pharmaceutical compositions comprising agents which are both EP<sub>2</sub> receptor selective and EP<sub>4</sub> receptor selective.

[0002] Osteoporosis is a systemic skeletal disease, characterized by low bone mass and deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture. In the U.S., the condition affects more than 25 million people and causes more than 1.3 million fractures each year, including 500,000 spine, 250,000 hip and 240,000 wrist fractures annually. Hip fractures are the most serious consequence of osteoporosis, with 5-20% of patients dying within one year, and over 50% of survivors being incapacitated.

[0003] The elderly are at greatest risk of osteoporosis, and the problem is therefore predicted to increase significantly with the aging of the population. Worldwide fracture incidence is forecasted to increase three-fold over the next 60 years, and one study has estimated that there will be 4.5 million hip fractures worldwide in 2050.

[0004] Women are at greater risk of osteoporosis than men. Women experience a sharp acceleration of bone loss during the five years following menopause. Other factors that increase the risk include smoking, alcohol abuse, a sedentary lifestyle and low calcium intake.

[0005] There are currently two main types of pharmaceutical therapy for the treatment of osteoporosis. The first is the use of anti-resorptive compounds to reduce the resorption of bone tissue.

[0006] Estrogen is an example of an anti-resorptive agent. It is known that estrogen reduces fractures. In addition, Black, et al. in EP 0605193A1 report that estrogen, particularly when taken orally, lowers plasma levels of LDL and raises those of the beneficial high density lipoproteins (HDL's). However, estrogen failed to restore bone back to young adult levels in the established osteoporotic skeleton. Furthermore, long-term estrogen therapy has been implicated in a variety of disorders including an increase in the risk of uterine cancer, endometrial cancer and possibly breast cancer, causing many women to avoid this treatment. The significant undesirable effects associated with estrogen therapy support the need to develop alternative therapies for osteoporosis that have the desirable effect on serum LDL but do not cause undesirable effects.

[0007] A second type of pharmaceutical therapy for the treatment of osteoporosis is the use of anabolic agents to promote bone formation and increase bone mass. This class of agents is expected to restore bone to the established osteoporotic skeleton.

[0008] U.S. pat. no. 4,112,236 discloses certain triphenylene 8-aza-9-dioxathia-11,12-seco prostaglandins for the treatment of patients with renal impairment.

[0009] Certain prostaglandin agonists are disclosed in GB 1478281, GB1479156 and U.S. pat. nos. 4,175,203; 4,055,598; 4,175,203; 3,987,091 and 3,991,106 as being useful as, for example, renal vasodilators.

[0010] U.S. pat. no. 4,033,996 discloses certain 8-aza-9-oxa (and dioxo)-thia-11,12-seco prostaglandins which are useful as renal vasodilators, for the prevention of thrombus formation, to induce growth hormone release, and as regulators of the immune response.

[0011] French patent no. 857,586 discloses certain amino acid derivatives for the treatment of neurological, mental or cardiovascular disease.

[0012] U.S. pat. no. 4,761,430 discloses certain arylbenzenesulfonamide compounds as lipid-lowering agents.

[0013] U.S. pat. no. 4,443,477 discloses certain subphosphorylphenylcarboxylic acids as lipid lowering agents.

[0014] U.S. pat. no. 3,528,861 discloses certain  $\epsilon$ -caprolactam derivatives as dyes.

[0015] U.S. pat. no. 3,780,095 discloses certain acylated anilinoalkanoic acids as choleretics.

[0016] U.S. pat. no. 4,243,878 discloses certain acylhydrocarbylaminoalkanoic acids as having utility in the treatment of gastric ulcers, as sebaceous gland excretion inhibitors and for combating skin inflammation.

[0017] U.S. pat. no. 4,386,031 discloses certain N-benzoyl- $\alpha$ -arilinoalkanoic acids as antidiabetic agents, thrombotic aggregation inhibitors, antinflammatory agents and lipid-lowering agents.

[0018] In addition to osteoporosis, approximately 20-25 million women and an increasing number of men have detectable vertebral fractures as a consequence of reduced bone mass, with an additional 250,000 hip fractures reported yearly in America alone. This latter case is associated with a 12% mortality rate within the first two years and with a 30% rate of patients requiring nursing home care after the fracture. While this is already significant, the economic and medical consequences of convalescence due to slow or imperfect healing of these bone fractures is expected to increase, due to the aging of the general population.

[0019] Estrogens have been shown (Boland et al., 38th Annual Meeting Orthopedic Research Society, 1992) to

improve the quality of the healing of appendicular fractures. Therefore, estrogen replacement therapy might appear to be a method for the treatment of fracture repair. However, patient compliance with estrogen therapy is relatively poor due to the side effects, including the resumption of menses, mastodynia, an increased risk of uterine cancer, an increased perceived risk of breast cancer, and the concomitant use of progestins. In addition, men are likely to object to the use of estrogen treatment. The need exists for a therapy which would be beneficial to patients who have suffered debilitating bone fractures and which would increase patient compliance.

[0020] Although there are a variety of osteoporosis therapies, there is a continuing need and a continuing search in this field of art for alternative osteoporosis therapies. In addition, there is a need for bone fracture healing therapies. Also, there is a need for therapy which can promote bone re-growth into skeletal areas where defects exist such as defects caused or produced by, for example, tumors in bone. Further, there is a need for therapy which can promote bone re-growth into skeletal areas where bone grafts are indicated.

#### SUMMARY OF THE INVENTION

[0021] This invention is directed to pharmaceutical compositions comprising an  $EP_2$  receptor selective agonist, a prodrug thereof or a pharmaceutically acceptable salt of said  $EP_2$  receptor selective agonist or of said prodrug, and an  $EP_4$  receptor selective agonist, a prodrug thereof or a pharmaceutically acceptable salt of said  $EP_4$  receptor selective agonist or of said prodrug. It is preferred that such pharmaceutical compositions additionally comprise a pharmaceutically acceptable vehicle, carrier or diluent.

[0022] This invention is also directed to methods of treating a condition which presents with low bone mass in a mammal comprising administering to said mammal a pharmaceutical composition as described above. In one of the preferred embodiments of the methods of this invention, the composition is administered systemically. In another preferred embodiment of the methods of this invention, the composition is administered locally. Conditions which present with low bone mass which are treated by the compositions, methods and kits of this invention include, but are not limited to, osteoporosis, osteoporotic fractures, bone defects, childhood idiopathic bone loss, alveolar bone loss, mandibular bone loss, bone fracture, osteotomy, bone loss associated with periodontitis and prosthetic ingrowth.

[0023] This invention is also directed to methods of treating a condition which presents with low bone mass in a mammal comprising administering to said mammal an  $EP_4$  receptor selective agonist, a prodrug thereof or a pharmaceutically acceptable salt of said  $EP_4$  receptor selective agonist or of said prodrug, and an  $EP_2$  receptor selective agonist, a prodrug thereof or a pharmaceutically acceptable salt of said  $EP_2$  receptor selective agonist, or of said prodrug.

[0024] This invention is especially directed to methods wherein the  $EP_4$  receptor selective agonist, prodrug thereof or pharmaceutically acceptable salt of said  $EP_4$  receptor selective agonist or of said prodrug, and the  $EP_2$  receptor selective agonist, prodrug thereof or pharmaceutically acceptable salt of said  $EP_2$  receptor selective agonist or of said prodrug, are administered separately and in any order.

[0025] This invention is also especially directed to methods wherein the  $EP_4$  receptor selective agonist, prodrug thereof or pharmaceutically acceptable salt of said  $EP_4$  receptor selective agonist or of said prodrug, and the  $EP_2$  receptor selective agonist, prodrug thereof or pharmaceutically acceptable salt of said  $EP_2$  receptor selective agonist or of said prodrug, are administered together.

[0026] This invention is also directed to methods of treating a condition which presents with low bone mass in a mammal comprising administering to said mammal an effective amount of an  $EP_2/EP_4$  receptor selective agonist, a prodrug thereof or a pharmaceutically acceptable salt of thereof or of said prodrug.

[0027] This invention is also directed to kits comprising:

- a first unit dosage form comprising an  $EP_2$  receptor selective agonist, a prodrug thereof or a pharmaceutically acceptable salt of said  $EP_2$  receptor selective agonist or of said prodrug, and a pharmaceutically acceptable vehicle or diluent;
- a second unit dosage form comprising an  $EP_4$  receptor selective agonist, a prodrug thereof or a pharmaceutically acceptable salt of said  $EP_4$  receptor selective agonist or of said prodrug, and a pharmaceutically acceptable vehicle or diluent; and
- a container.

[0028] Preferably post-menopausal women and men over the age of 50 are treated. Also preferred is treatment of individuals, regardless of age, who have significantly reduced bone mass, i.e., greater than or equal to 1.5 standard deviations below young normal levels.

[0029] Methods for treating "secondary osteoporosis" are also included within the methods of this invention. "Secondary osteoporosis" includes glucocorticoid-induced osteoporosis, hyperthyroidism-induced osteoporosis, immobilization-induced osteoporosis, heparin-induced osteoporosis and immunosuppressive-induced osteoporosis in a verte-

brate, e.g., a mammal (including a human being). Said treatment is achieved by administering to said vertebrate, e.g., a mammal, suffering from "secondary osteoporosis," a "secondary osteoporois" effective treating amount of a pharmaceutical composition comprising an  $EP_2/EP_4$  receptor selective agonist, a prodrug thereof or a pharmaceutically acceptable salt of said  $EP_2/EP_4$  receptor selective agonist or of said prodrug, or by administering to said vertebrate, e.g., a mammal, a "secondary osteoporois" treating effective amount of an  $EP_4$  receptor selective agonist, a prodrug thereof or a pharmaceutically acceptable salt of said  $EP_4$  receptor selective agonist or of said prodrug, and an  $EP_2$  receptor selective agonist, a prodrug thereof or a pharmaceutically acceptable salt of said  $EP_2$  receptor selective agonist or of said prodrug.

[0030] Yet another aspect of this invention is directed to methods for strengthening a bone graft, inducing vertebral osteosynthesis, enhancing long bone extension, enhancing bone healing following facial reconstruction, maxillary reconstruction or mandibular reconstruction in a vertebrate, e.g., a mammal (including a human being), comprising administering to said vertebrate, e.g., a mammal which has undergone facial reconstruction, maxillary reconstruction or mandibular reconstruction, a bone enhancing amount of a pharmaceutical composition comprising an  $EP_2/EP_4$  receptor selective agonist, a prodrug thereof or a pharmaceutically acceptable salt of said  $EP_2/EP_4$  receptor selective agonist, a prodrug thereof or a pharmaceutically acceptable salt of said  $EP_4$  receptor selective agonist or of said prodrug, and an  $EP_2$  receptor selective agonist, a prodrug thereof or a pharmaceutically acceptable salt of said  $EP_2$  receptor selective agonist or of said prodrug. Whether administered separately or together, the active agents of this invention may be applied locally to the site of bone reconstruction or may be administered systemically.

[0031] A preferred dosage is about 0.001 to 100 mg/kg/day of a Formula I compound, a prodrug thereof or a pharmaceutically acceptable salt of said compound or said prodrug. An especially preferred dosage is about 0.01 to 10 mg/kg/day of a Formula I compound, a prodrug thereof or a pharmaceutically acceptable salt of said compound or said prodrug.

[0032] The phrase "condition(s) which presents with low bone mass" refers to a condition where the level of bone mass is below the age specific normal as defined in standards by the World Health Organization "Assessment of Fracture Risk and its Application to Screening for Postmenopausal Osteoporosis" (1994). Report of a World Health Organization Study Group, World Health Organization Technical Series 843. Included in "condition(s) which presents with low bone mass" are primary and secondary osteoporosis. Secondary osteoporosis includes glucocorticoid-induced osteoporosis, hyperthyroidism-induced osteoporosis, immobilization-induced osteoporosis, heparin-induced osteoporosis and immunosuppressive-induced osteoporosis. Also included is periodontal disease, alveolar bone loss, post-osteotomy and childhood idiopathic bone loss. The phrase "condition(s) which presents with low bone mass" also includes long term complications of osteoporosis such as curvature of the spine, loss of height and prosthetic surgery.

[0033] The phrase "condition(s) which presents with low bone mass" also refers to a vertebrate, e.g., a mammal, known to have a significantly higher than average chance of developing such diseases as are described above including osteoporosis (e.g., post-menopausal women, and men over the age of 60).

[0034] Other bone mass augmenting or enhancing uses include bone restoration, increasing the bone fracture healing rate, replacing bone graft surgery entirely, enhancing the rate of successful bone grafts, bone healing following facial reconstruction or maxillary reconstruction or mandibular reconstruction, prosthetic ingrowth, vertebral osteosynthesis and long bone extension.

[0035] The compounds and compositions of this invention may also be used in conjunction with orthopedic devices such as spinal fusion cages, spinal fusion hardware, internal and external bone fixation devices, screws and pins.

[0036] Those skilled in the art will recognize that the term bone mass actually refers to bone mass per unit area which is sometimes (although not strictly correctly) referred to as bone mineral density.

[0037] The term "treating", "treat" or "treatment" as used herein includes preventative (e.g., prophylactic), palliative and curative treatment.

[0038] By "pharmaceutically acceptable" it is meant the carrier, vehicle, diluent, excipients, and/or salt must be compatible with the other ingredients of the formulation, and not deleterious to the recipient thereof.

[0039] The expression "prodrug" refers to a compound that is a drug precursor which, following administration, releases the drug *in vivo* via some chemical or physiological process (e.g., a prodrug on being brought to the physiological pH or through enzyme action is converted to the desired drug form). Exemplary prodrugs upon cleavage release the corresponding drug compounds.

[0040] The expression "pharmaceutically acceptable salt" refers to nontoxic anionic salts containing anions such as (but not limited to) chloride, bromide, iodide, sulfate, bisulfate, phosphate, acetate, maleate, fumarate, oxalate, lactate, tartrate, citrate, gluconate, methanesulfonate and 4-toluene-sulfonate. The expression also refers to nontoxic cationic salts such as (but not limited to) sodium, potassium, calcium, magnesium, ammonium or protonated benzathine (N, N'-dibenzylethylenediamine), choline, ethanolaniline, diethanolamine, ethylenediamine, N-methylglucamine, benethamine (N-benzylphenethylamine), piperazine and tromethamine (2-amino-2-hydroxyethyl-1,3-propanediol).



each optionally substituted with up to four substituents each independently selected from fluoro or  $(C_1-C_3)alkyl$ ,  $-(C_1-C_3)alkylene$ -ethynylene,  $-(C_1-C_3)alkylene$ -X-W $-(C_1-C_3)alkylene$ , said alkylenes and said ethynylene each optionally substituted with up to four substituents each independently selected from fluoro or  $(C_1-C_3)alkyl$ .

$C_2$ - $C_3$  alkylene-ethynylene- $(C_1-C_4)$  alkylene-, said alkynes and said ethynylene each optionally substituted with up to four substituents each independently selected from fluoro or  $(C_1-C_4)$ alkyl, or  $(C_1-C_4)$  alkylene-ethynylene- $X-(C_2-C_3)$ alkylene-, said alkynes and said ethynylene each optionally substituted with up to four substituents each independently selected from fluoro or  $(C_1-C_4)$ alkyl;

1,2,4-thiadiazolyl, (C<sub>1</sub>-C<sub>6</sub>)alkylsulfonylcarbomoyl or phenylsulfonylcarbomoyl;

[illegible]

M is  $\text{Ar}^3$ ,  $\text{Ar}^1\text{Ar}^1\text{Ar}^1$ ,  $\text{Ar}^1\text{Ar}^1\text{Ar}^2$ ,  $\text{Ar}^1\text{Ar}^1\text{Ar}^3$ ,  $\text{Ar}^1\text{Ar}^2\text{Ar}^2$ ,  $\text{Ar}^1\text{Ar}^2\text{Ar}^3$ ,  $\text{Ar}^1\text{Ar}^3\text{Ar}^3$ ,  $\text{Ar}^2\text{Ar}^2\text{Ar}^2$ , or  $\text{Ar}^2\text{Ar}^2\text{Ar}^3$ . Ar is a partially saturated or fully unsaturated five- to eight-membered ring optionally having one to four heteroatoms selected independently from oxygen, sulfur and nitrogen, or a bicyclic ring consisting of two fused five- to eight-membered rings, taken independently from oxygen, sulfur and nitrogen, or a bicyclic ring consisting of two fused five- or six-membered rings, taken independently partially saturated, fully saturated or fully unsaturated five- or six-membered rings, taken independently, optionally having one to four heteroatoms selected independently from nitrogen, sulfur and oxygen, or a bicyclic ring consisting of three fused independently partially saturated, fully saturated or fully unsaturated five- or six-membered rings, taken independently, optionally having one to four heteroatoms selected independently from nitrogen, sulfur and oxygen, said partially or fully saturated ring, bicyclic ring or tricyclic ring optionally having one or two oxo groups substituted on carbon or one or two oxo groups substituted on sulfur, or Ar is a fully saturated five- to seven-membered ring having one or two heteroatoms selected independently from oxygen, sulfur and nitrogen.

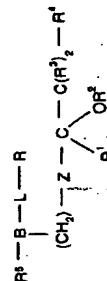
the A1 and A2<sub>2</sub> are each independently a partially saturated, fully saturated or fully unsaturated five-, to eight-membered ring optionally having one to four heteroatoms selected independently from oxygen, sulfur and nitrogen, or a bicyclic ring consisting of two fused independently partially saturated, fully saturated or fully unsaturated five- or six-membered rings, taken independently, optionally having one to four heteroatoms selected independently from oxygen, sulfur and nitrogen, or a bicyclic ring consisting of three fused independently unsaturated five- or six-membered rings, and a bicyclic ring consisting of two fused independently partially saturated, fully saturated or fully unsaturated five- or six-membered rings, optionally having one to four heteroatoms selected independently from nitrogen, sulfur and oxygen, said partially or fully saturated ring, bicyclic ring or bicyclic ring optionally having one or two oxo groups substituted on carbon or one or two oxo groups substituted on sulfur;

[illegible][illegible][illegible][illegible][illegible]

X is a five- or six-membered aromatic ring optionally having one or two heteroatoms selected independently from oxygen, nitrogen, and sulfur; said ring optionally mono-, di- or tri-substituted independently with halo, alkyl, alkenyl, aryl, trifluoromethyl, trifluoromethoxy, difluoromethoxy, hydroxyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxy, or carbamoyl; R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, R<sup>8</sup>, R<sup>9</sup>, R<sup>10</sup>, R<sup>11</sup>, R<sup>12</sup>, R<sup>13</sup>, R<sup>14</sup>, and R<sup>15</sup> are each independently an alkyl, alkenylene, alkynylene or alkoxyalkenylene moiety.

[illegible]

(iii) compounds of Formula III



### Formula III

prodrugs thereof, and the pharmaceutically acceptable salts of the compounds and the prodrugs, wherein:

B is N or C(Q'), where Q' is H or (C<sub>1</sub>-C<sub>3</sub>)alkyl;

L is n-propylphenyl-X- or CH<sub>2</sub>-melaphenylene-CH<sub>2</sub>, wherein X is (uranyl), thieryl, thiazoyl or tetrahydrofuranyl, or tri-substituted aromatic carbon independent CH<sub>2</sub>-melaphenylene-CH<sub>2</sub> or X being optionally mono-, di- or tri-substituted on aromatic carbon independent CH<sub>2</sub>-melaphenylene-CH<sub>2</sub> or X being optionally fluoro, methoxy, difluoromethoxy, trifluoromethyl or methyl;

R is carboxyl, ( $C_1-C_{10}$ )alkoxycarbonyl, tetrazolyl, 5-oxo-1,2,4-thiadiazolyl, 5-oxo-1,2,4-oxadiazolyl, ( $C_1-C_{10}$ )alkylsulfonylcarbonyl or phenylsulfonylcarbonyl;

R<sup>1</sup> is H, methyl, ethyl or propyl;

R<sup>2</sup> is H or (C<sub>1</sub> - C<sub>10</sub>) alkenyl;

$H^1$  is  $H^1(C_2 - C_3)$  algebraically,  
 $H^2$  is independently  $H^1$  algebraically or mathematically.

$R^4$  is independently  $H$ , fluoro or methyl;  
 $R^5$  is independently  $H$ , fluoro or methyl;  
 $R^6$  is  $H$ ,  $(C_1 - C_3)$  alkyl, or  $R^4$  and  $R^5$  are taken together to form a 5-9 membered carbocyclic ring, said alkyl being optionally monosubstituted and optionally mono-, di- or tri-substituted independently with one to three fluoro, chloro, methoxy, difluoromethoxy, trifluoromethoxy, trifluoromethyl or methyl;

[illegible]

Z is methylene, ethylene, propylene or ethenylene;

[illegible]



(3-((pyridine-3-sulfonyl)-(4-pyrimidin-5-yl)-benzyl)-amino)-methyl)-phenyl)-acetic acid;  
 (3-((5-phenyl-luran-2-ylmethyl)-(pyridine-3-sulfonyl)-amino)-methyl)-phenyl)-acetic acid;  
 (3-((pyridine-3-sulfonyl)-(4-pyrimidin-2-yl)-benzyl)-amino)-methyl)-phenyl)-acetic acid;  
 (3-((pyridine-3-sulfonyl)-(4-thiazol-2-yl)-benzyl)-amino)-methyl)-phenyl)-acetic acid;  
 (3-((4-pyrazin-2-yl)-benzyl)-(pyridine-3-sulfonyl)-amino)-methyl)-phenyl)-acetic acid;  
 (3-((4-cyclohexyl)-benzyl)-(pyridine-3-sulfonyl)-amino)-methyl)-phenyl)-acetic acid;  
 (3-((pyridine-3-sulfonyl)-(4-pyridin-2-yl)-benzyl)-amino)-methyl)-phenyl)-acetic acid;  
 (3-((pyridine-3-sulfonyl)-(4-pyridin-3-yl)-benzyl)-amino)-methyl)-phenyl)-acetic acid;  
 (3-((pyridine-3-sulfonyl)-(4-pyridin-4-yl)-benzyl)-amino)-methyl)-phenyl)-acetic acid;  
 (3-((pyridine-3-sulfonyl)-(4-pyridin-5-yl)-benzyl)-amino)-methyl)-phenyl)-acetic acid;  
 (3-((2,3-dihydro-benz[1,4]dioxin-6-ylmethyl)-(pyridine-3-sulfonyl)-amino)-methyl)-phenyl)-acetic acid;  
 (3-((2,3-dichloro-phenyl)-thiazol-2-yl)-benzyl)-amino)-methyl)-phenyl)-acetic acid;  
 (3-((4-butyl)-benzyl)-(pyridine-3-sulfonyl)-amino)-methyl)-phenyl)-acetic acid;  
 (3-((benzenesulfonyl)-(4-butyl)-benzyl)-amino)-methyl)-phenyl)-acetic acid;  
 (3-((4-butyl)-benzyl)-(1-methyl-1H-imidazol-4-sulfonyl)-amino)-methyl)-phenyl)-acetic acid;  
 (3-((4-dimethylamino)-benzyl)-(pyridine-3-sulfonyl)-amino)-methyl)-phenyl)-acetic acid;  
 (3-((4-dimethylamino)-benzyl)-(pyridine-3-sulfonyl)-amino)-methyl)-phenyl)-acetic acid;  
 (3-((4-tert-butyl)-benzyl)-(pyridine-3-sulfonyl)-amino)-methyl)-phenyl)-acetic acid;  
*trans*-3-((3-((3,5-dichloro-phenyl)-allyl)-(pyridine-3-sulfonyl)-amino)-methyl)-phenyl)-acetic acid;  
 (3-((2,3-dichloro-phenyl)-ethyl)-(pyridine-3-sulfonyl)-amino)-methyl)-phenyl)-acetic acid; the prodrugs thereof, and the pharmaceutically acceptable salts of the compounds, and the prodrugs.

[0046] A preferred subgroup of Formula III comprises those compounds wherein:

B is N; R is carboxyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxycarbonyl or tetrazolyl; Z is ethylenyl; R<sup>1</sup> and R<sup>2</sup> are each H; and L is CH<sub>2</sub> metaphenylene-CH<sub>2</sub> or n-propylene-X; the prodrugs thereof, and the pharmaceutically acceptable salts of the compounds, and the prodrugs.

A further preferred subgroup of Formula III comprises those compounds wherein:

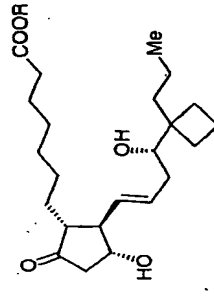
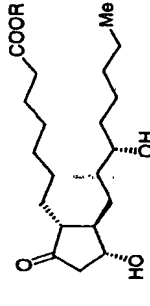
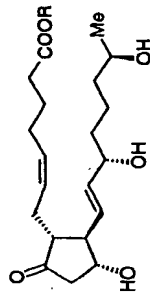
R<sup>1</sup> is selected from (C<sub>1</sub>-C<sub>6</sub>)alkylcarbonyl, optionally mono-, di-, or tri-substituted with hydroxy or fluoro; (C<sub>1</sub>-C<sub>6</sub>)alkylsulfonyl or (C<sub>2</sub>-C<sub>6</sub>)cycloalkylsulfonyl; and G-sulfonyl, wherein G is phenyl, imidazolyl, pyridyl, pyrazolyl, or pyrimidinyl optionally mono-, di-, or tri-substituted on carbon or nitrogen with chloro, fluoro, methoxy, difluoromethoxy, trifluoromethoxy, trifluoromethyl or methyl; the prodrugs thereof, and the pharmaceutically acceptable salts of the compounds, and the prodrugs.

[0047] A preferred subgroup of Formula IV comprises those compounds selected from:

*trans*-7-[2-(2-(3,5-bis-(trifluoromethyl)-phenyl)-vinyl)-5-oxo-cyclopentyl]-heptanoic acid;  
*trans*-7-[2-(2-(4-chloro-3-trifluoromethyl-phenyl)-vinyl)-5-oxo-cyclopentyl]-heptanoic acid;  
*trans*-7-[2-(2-(3,5-dichlorophenyl)-vinyl)-5-oxo-cyclopentyl]-heptanoic acid;  
*trans*-7-[2-(2-(3,5-dichlorophenyl)-vinyl)-5-oxo-cyclopentyl]-heptanoic acid;  
*trans*-7-[2-(2-(3,5-dichlorophenyl)-vinyl)-5-oxo-cyclopentyl]-heptanoic acid;  
*trans*-7-[2-(2-(4-fluoro-phenyl)-vinyl)-5-oxo-cyclopentyl]-heptanoic acid;  
*trans*-7-[2-(2-(3,5-bis-(trifluoromethyl)-phenyl)-vinyl)-5-oxo-cyclopentyl]-heptanoate;  
*trans*-7-[2-(2-(4-chloro-3-trifluoromethyl-phenyl)-vinyl)-5-oxo-cyclopentyl]-heptanoate;  
*trans*-7-[2-(2-(3,5-dichlorophenyl)-vinyl)-5-oxo-cyclopentyl]-heptanoate;  
*trans*-7-[2-(2-(3,5-dichlorophenyl)-vinyl)-5-oxo-cyclopentyl]-heptanoate;  
*trans*-7-[2-(2-(3,5-dichlorophenyl)-vinyl)-5-oxo-cyclopentyl]-heptanoate;  
*trans*-7-[2-(2-(4-fluoro-phenyl)-vinyl)-5-oxo-cyclopentyl]-heptanoate;  
*trans*-3-[2-(3,5-bis-(trifluoromethyl)-phenyl)-vinyl]-2-[6-(2H-tetrazol-5-yl)-hexyl]-cyclopentanone;  
*trans*-3-[2-(4-chloro-3-trifluoromethyl-phenyl)-vinyl]-2-[6-(2H-tetrazol-5-yl)-hexyl]-cyclopentanone;  
*trans*-3-[2-(3,5-dichloro-phenyl)-vinyl]-2-[6-(2H-tetrazol-5-yl)-hexyl]-cyclopentanone;  
*trans*-3-[2-(3,5-dichloro-phenyl)-vinyl]-2-[6-(2H-tetrazol-5-yl)-hexyl]-cyclopentanone;  
*trans*-3-[2-(3,5-dichloro-phenyl)-vinyl]-2-[6-(2H-tetrazol-5-yl)-hexyl]-cyclopentanone;  
*trans*-3-[2-(3,5-dichloro-phenyl)-vinyl]-2-[6-(2H-tetrazol-5-yl)-hexyl]-cyclopentanone;  
*trans*-3-[2-(4-fluoro-phenyl)-vinyl]-2-[6-(2H-tetrazol-5-yl)-hexyl]-cyclopentanone; the prodrugs thereof, and the pharmaceutically acceptable salts of the compounds, and the prodrugs.

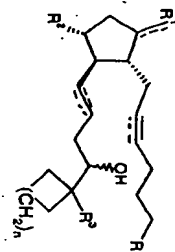
[0048] The compounds of Formula I, the prodrugs thereof, and the pharmaceutically acceptable salts of the compounds and the prodrugs, may be prepared according to the synthetic methodologies described in International Patent

Application Publication No. WO 98/28264.  
 [0049] The compounds of Formula II, the prodrugs thereof, and the pharmaceutically acceptable salts of the compounds and the prodrugs, may be prepared according to the synthetic methodologies described in International Application Patent Publication No. WO 99/19300.  
 [0050] The compounds of Formula III, the prodrugs thereof, and the pharmaceutically acceptable salts of the compounds and the prodrugs, may be prepared according to the synthetic methodologies described in European Patent Application Publication No. EP 0 911 321.  
 [0051] The compounds of Formula IV, the prodrugs thereof, and the pharmaceutically acceptable salts of the compounds and the prodrugs, may be prepared according to the synthetic methodologies described in International Patent Application Publication No. WO 99/58811.  
 [0052] Other EP<sub>2</sub> receptor selective agonists which may be used in the compositions, methods and kits of this invention include compounds of the formula

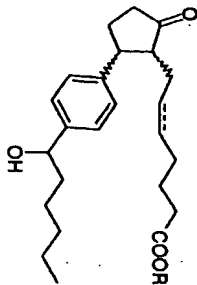


wherein the R is defined, and the compounds are prepared, as disclosed in U.S. Patent No. 5,698,598, which is incorporated herein by reference.

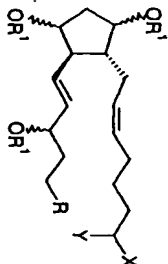
[0053] Yet other EP<sub>2</sub> receptor selective agonists which may be used in the compositions, methods and kits of this invention include compounds of the formula



wherein the various substituents are defined, and the compounds are prepared, as disclosed in European Patent Application Publication No. EP 0 680 430, which is incorporated herein by reference.  
[0054] Still other EP<sub>2</sub> receptor selective agonists which may be used in the compositions, methods and kits of this invention include compounds of the formula



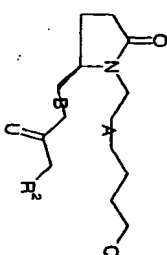
wherein the various substituents are defined, and the compounds are prepared, as disclosed in International Patent Application Publication No. WO95/18984, which is incorporated herein by reference.  
[0055] Further EP<sub>2</sub> receptor selective agonists which may be used in the compositions, methods and kits of this invention include compounds of the formula



wherein the various substituents are defined, and the compounds are prepared, as disclosed in International Patent Application Publication No. WO98/25358, which is incorporated herein by reference.  
[0056] Any EP<sub>4</sub> receptor selective agonist may be used as the EP<sub>4</sub> receptor selective agonist of this invention. Preferred EP<sub>4</sub> receptor selective agonists include compounds of Formula V<sub>1</sub>:

55

13



prodrugs thereof and pharmaceutically acceptable salts of said compounds or said prodrugs, wherein:

O is COOR', CONHR' or tetrazol-5-yl;

A is a single or cis double bond;

B is a single or trans double bond;

U is

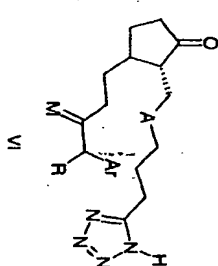


R<sup>1</sup> is α-allyl, phenyl, phenoxy, monosubstituted phenyl and monosubstituted phenoxy, said substituents being chloro, fluoro, phenyl, methoxy, trifluoromethyl or (C<sub>1</sub>-C<sub>3</sub>)alkyl;  
R<sup>2</sup> is hydrogen, (C<sub>1</sub>-C<sub>3</sub>)alkyl, phenyl or p-biphenyl;  
R<sup>3</sup> is COR<sup>4</sup> or SO<sub>2</sub>R<sup>5</sup>; and  
R<sup>4</sup> is phenyl or (C<sub>1</sub>-C<sub>3</sub>)alkyl.

[0057] A preferred group of EP<sub>4</sub> receptor selective agonists of Formula V are those compounds of Formula V wherein O is 5-tetrazolyl. Particularly preferred compounds within this group include 5-(3-hydroxy-4-phenyl-but-1-enyl)-1-(6-(1H-tetrazol-5-yl)-hexyl)-pyrrolidin-2-one and 5-(3-hydroxy-4-phenyl-butyl)-1-(6-(1H-tetrazol-5-yl)-hexyl)-pyrrolidin-2-one.

[0058] Another preferred group of EP<sub>4</sub> receptor selective agonists of Formula V are those compounds of Formula V wherein O is COOH. Particularly preferred compounds within this group include 7-(2-(3-hydroxy-4-phenyl-butyl)-5-oxo-pyrrolidin-1-yl)-heptanoic acid and 7-(2-(3-hydroxy-4-phenyl-butyl)-5-oxo-pyrrolidin-1-yl)-heptanoic acid.

[0059] Any EP<sub>2</sub>/EP<sub>4</sub> receptor selective agonist may be used as the EP<sub>2</sub>/EP<sub>4</sub> receptor selective agonist of this invention. Preferred EP<sub>2</sub>/EP<sub>4</sub> receptor selective agonists are compounds of the Formula VI:

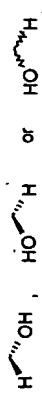


wherein A is ethylene or cis-vinylene, M is

14



R is hydrogen or methyl; and Ar is phenyl, wherein said phenyl is optionally monosubstituted with fluoro, chloro, bromo, trifluoromethyl, methyl, methoxy or phenyl. Such compounds are prepared as described in U.S. Patent No. 4,097,801, which is incorporated herein by reference. Particularly preferred  $\text{EP}_2/\text{EP}_4$  receptor selective agonist is the compound of the Formula VI wherein A is ethylene, M is



R is hydrogen, and Ar is phenyl, i.e., 3-(3-hydroxy-4-phenyl-butyl)-2-[(E)-(1 H-tetrazol-5-yl)-hexyl]-cyclopentanone. [0060] The compositions of this invention are all adapted to therapeutic use as agents that stimulate bone formation and increase bone mass in vertebrates, e.g., mammals, and particularly humans. Since bone formation is closely related to the development of osteoporosis and bone related disorders, these compositions, by virtue of their action on bone, prevent, arrest and/or regress osteoporosis.

[0061] The utility of the compositions of the present invention as medical agents in the treatment of conditions which present with low bone mass (e.g., osteoporosis) in vertebrates, e.g., mammals (especially humans and particularly female humans) is demonstrated by the activity of the compositions of this invention in conventional assays, including a receptor binding assay, a cyclic AMP assay, an *in vivo* assay and a fracture healing assay (all of which are described below). Such assays also provide a means whereby the activities of the compositions of this invention can be compared to each other and with the activities of other known compounds and compositions. The results of these comparisons are useful for determining dosage levels in a vertebrate, e.g., mammal, including humans, for the treatment of such diseases.

#### Analgesic Agent *In Vivo* Assay

[0062] The activity of analgesic bone agents in stimulating bone formation and increasing bone mass can be tested in intact male or female rats, sex hormone deficient male (orchidectomized) or female (ovariectomized) rats.

[0063] Male or female rats at different ages (such as 3 months of age) can be used in the study. The rats are either intact or castrated (ovariectomized or orchidectomized), and subcutaneously injected or gavage with prostaglandin agonists at different doses (such as 1, 3, or 10 mg/kg/day) for 30 days. In the castrated rats, treatment is started at the next day after surgery (for the purpose of preventing bone loss) or at the time bone loss has already occurred (for the purpose of restoring bone mass). During the study, all rats are allowed free access to water and a pelleted commercial diet (Teklad Rodent Diet #8064, Harlan Teklad, Madison, WI) containing 1.46% calcium, 0.99% phosphorus and 4.36 IU/g of Vitamin D<sub>3</sub>. All rats are given subcutaneous injections of 10 mg/kg calcitonin on days 12 and 2 before sacrifice. The rats are sacrificed. The following endpoints are determined:

#### Femoral Bone Mineral Measurements:

[0064] The right femur from each rat is removed at autopsy and scanned using dual energy X-ray absorptiometry (DXA, QDR 1000W, Hologic Inc., Waltham, MA) equipped with "Regional High Resolution Scan" software (Hologic Inc., Waltham, MA). The scan field size is 5.08 x 1.802 cm, resolution is 0.0254 x 0.0127 cm and scan speed is 7.25 mm/second. The femoral scan images are analyzed and bone area, bone mineral content (BMC), and bone mineral density (BMD) of whole femora (WF), distal femoral metaphyses (DFM), femoral shaft (FS), and proximal femora (PF) are determined.

#### Tibial Bone Histomorphometric Analyses:

[0065] The right tibia is removed at autopsy, dissected free of muscle, and cut into three parts. The proximal tibia and the tibial shaft are fixed in 70% ethanol, dehydrated in graded concentrations of ethanol, dehydrated in acetone, then embedded in methyl methacrylate (Eastman Organic Chemicals, Rochester, NY). [0066] Frontal sections of proximal tibial metaphyses at 4 and 10 µm thickness are cut using a Reichert-Jung Polycut S microtome. The 4 µm sections are stained with modified Masson's Trichrome stain while the 10 µm sections remained unstained. One 4 µm and one 10 µm sections from each rat are used for cancellous bone histomorphometry.

[0067] Cross sections of tibial shaft at 10 µm thickness are cut using a Reichert-Jung Polycut S microtome. These sections are used for cortical bone histomorphometric analysis.

[0068] Cancellous bone histomorphometry: A Bloquant/OS2 histomorphometry system (RAM Biometrics, Inc., Nashvile, TN) is used for the static and dynamic histomorphometric measurements of the secondary spongiosa of the proximal tibial metaphyses between 1.2 and 3.6 mm distal to the growth plate-epiphyseal junction. The first 1.2 mm of the tibial metaphyseal region needs to be omitted in order to restrict measurements to the secondary spongiosa. The 4 µm sections are used to determine indices related to bone volume, bone structure, and bone resorption, while the 10 µm sections are used to determine indices related to bone formation and bone turnover.

[0069] I. Measurements and calculations related to trabecular bone volume and structure: (1) Total metaphyseal area (TV, mm<sup>2</sup>); metaphyseal area between 1.2 and 3.6 mm distal to the growth plate-epiphyseal junction. (2) Trabecular bone area (BV, mm<sup>2</sup>); total area of trabeculae within TV. (3) Trabecular bone perimeter (BS, mm); the length of total perimeter of trabeculae. (4) Trabecular bone volume (BV/TV, %); BV / TV x 100. (5) Trabecular bone number (TbN, #/mm); 1.189 / (2 x BS /TV). (6) Trabecular bone thickness (TbT, µm); (2000/1.189) x (BV / BS). (7) Trabecular bone separation (TbS, µm); (2000 x 1.199) x (TV - BV).

[0070] II. Measurements and calculations related to bone resorption: (1) Osteoclast number (OCN, #); total number of osteoclast within total metaphyseal area. (2) Osteoclast perimeter (OCP, mm); length of trabecular perimeter covered by osteoclast. (3) Osteoclast number/mm (OCN/mm, #/mm); OCN / BS. (4) Percent osteoclast perimeter (%OCP, %); OCP / BS x 100.

[0071] III. Measurements and calculations related to bone formation and turnover: (1) Single-calcin labeled perimeter (LSL, mm); total length of trabecular perimeter labeled with one calcin label. (2) Double-calcin labeled perimeter (DLS, mm); total length of trabecular perimeter labeled with two calcin labels. (3) Inter-labeled width (ILW, µm); average distance between two calcin labels. (4) Percent mineralizing perimeter (PMS, %); (LSL2 + DLS) / BS x 100. (5) Mineral apposition rate (MAR, µm/day); ILW / label interval. (6) Bone formation rate/surface area (BF/BS, µm<sup>2</sup>/day); (LSL2 + DLS) x MAR / BS. (7) Bone turnover rate (BTR, %/yr); (LSL2 + DLS) x MAR / BV x 100.

[0072] Cortical bone histomorphometry: A Bloquant/OS2 histomorphometry system (RAM Biometrics, Inc., Nashvile, TN) is used for the static and dynamic histomorphometric measurements of tibial shaft cortical bone. Total tissue area, marrow cavity area, periosteal perimeter, endocortical perimeter, single labeled perimeter, double labeled perimeter, and interlabeled width on both periosteal and endocortical surfaces are measured, and cortical bone area (total tissue area - marrow cavity area), percent cortical bone area (cortical area / total tissue area x 100), percent marrow area (marrow cavity area / total tissue area x 100), periosteal and endocortical percent labeled perimeter (single labeled perimeter/2-double labeled perimeter) / total perimeter x 100, mineral apposition rate (interlabeled width / intervals), and bone formation rate [mineral apposition rate x (single labeled perimeter/2-double labeled perimeter) / total perimeter] are calculated.

#### Statistics

[0073] Statistics can be calculated using StatView 4.0 packages (Abacus Concepts, Inc., Berkeley, CA). The analysis of variance (ANOVA) test followed by Fisher's PLSD (Stat View, Abacus Concepts Inc., 1918 Bonita Ave, Berkeley, CA 94704-1014) are used to compare the differences between groups.

#### Determination of cAMP Elevation in 293-S Cell Lines Stably Overexpressing Recombinant Human $\text{EP}_2$ and $\text{EP}_4$ Receptors.

[0074] cDNAs representing the complete open reading frames of the human  $\text{EP}_2$  and  $\text{EP}_4$  receptors are generated by reverse transcriptase polymerase chain reaction using oligonucleotide primers based on published sequences (1, 2) and RNA from primary human kidney cells ( $\text{EP}_2$ ) or primary human lung cells ( $\text{EP}_4$ ) as templates. cDNAs are cloned into the multiple cloning site of pCDNA3 (Invitrogen Corporation, 395B Sorrento Valley Blvd., San Diego, CA 92121) and used to transfect 293-S human embryonic kidney cells via calcium phosphate co-precipitation. G418-resistant colonies are expanded and tested for specific [ $^3\text{H}$ ]PGE<sub>2</sub> binding. Transfectants demonstrating high levels of specific [ $^3\text{H}$ ]PGE<sub>2</sub> binding are further characterized by Scatchard analysis to determine Bmax and Kds for PGE<sub>2</sub>. The lines selected for compound screening have approximately 338,400 receptors per cell and a Kd = 12 nM for PGE<sub>2</sub> ( $\text{EP}_2$ ), and approximately 258,400 receptors per cell and a Kd = 2.8 nM for PGE<sub>2</sub> ( $\text{EP}_4$ ). Constitutive expression of both receptors in parental 293-S cells is negligible. Cells are maintained in RPMI supplemented with fetal bovine serum (10% final) and G418 (700 µg/ml final).

[0075] cAMP responses in the 293-S- $\text{EP}_2$  and 293-S- $\text{EP}_4$  lines are determined by detaching cells from culture flasks in 1 ml of Ca++ and Mg++ deficient PBS via vigorous pounding, adding serum-free RPMI to a final concentration of 1 x 10<sup>6</sup> cells/ml, and adding 3-isobutyl-1-methylxanthine (IBMX) to a final concentration of 1 mM. One milliliter of cell suspension is immediately aliquoted into individual 2 ml screwcap microcentrifuge and incubated for 10 minutes, un-



covered, at 37 °C, 5% CO<sub>2</sub>-95% relative humidity. The compound is then added to cells at 1:100 dilutions such that final DMSO or ethanol concentrations is 1%, immediately after adding compound the tubes are covered, mixed by inverting two times, and incubated at 37 °C for 12 minutes. Samples are then lysed by incubation at 100 °C for 10 minutes and immediately cooled on ice for 5 minutes. Cellular debris is pelleted by centrifugation at 1000 X g for 5 minutes, and cleared lysates are transferred to fresh tubes. cAMP concentrations are determined using a commercially available cAMP radioimmunoassay kit RIA (NEK-023, DuPont/NEN Research Products, 549 Albany St., Boston, MA 02118) after diluting cleared lysates 1:10 in cAMP RIA assay buffer (included in kit). Typically, one treat cells with 6-8 concentrations of the compound to be tested in 1 log increments. EC50 calculations are performed on a calculator using linear regression analysis on the linear portion of the dose response curves.

#### References

- [0076] 1. Regan, J.W., Bailey, T.J., Pappert, D.J., Pierce, K.L., Bogardus A.M., Donallo, J.E., Fairman, C.E., Kedzie, K.M., Woodward, D.F. and Gil, D.W. 1994 Cloning of a Novel Human Prostaglandin Receptor with Characteristics of the Pharmacologically Defined EP<sub>2</sub> Subtype. *Mol. Pharmacology* 46:213-220.
2. Basille, L., Sawyer, N., Grygorczyk, R., Matras, K., and Adam, M. 1994 Cloning, Functional Expression, and Characterization of the Human Prostaglandin EP<sub>2</sub> Receptor EP<sub>2</sub> Subtype. *J. Biol. Chem.* Vol.269, 18:11873-11877.

#### Assay for Binding to Prostaglandin E<sub>2</sub> Receptors

- [0077] Membrane Preparation: All operations are performed at 4 °C. Transfected cells expressing prostaglandin E<sub>2</sub> type 1 receptors (EP<sub>1</sub>), type 2 (EP<sub>2</sub>), type 3 (EP<sub>3</sub>) or type 4 (EP<sub>4</sub>) receptors are harvested and suspended to 2 million cells per ml in Buffer A (50 mM Tris-HCl (pH 7.4), 10 mM MgCl<sub>2</sub>, 1 mM EDTA, 1 mM Pefabloc peptide (Boehringer Mannheim Corp., Indianapolis, IN), 10 μM Phosphoramidon peptide (Sigma, St. Louis, MO), 1 μM pepstatin A peptide, (Sigma, St. Louis, MO), 10 μM elastatinal peptide (Sigma, St. Louis, MO), 100 μM aprotinin peptide (Sigma, St. Louis, MO). The cells are lysed by sonification with a Branson Sonifier (Model 4250, Branson Ultrasonics Corporation, Danbury, CT) in 2 fifteen second bursts. Unlysed cells and debris are removed by centrifugation at 100 X g for 10 min. Membranes are then harvested by centrifugation at 45,000 X g for 30 minutes. Pelleted membranes are resuspended to 3-10 mg protein per ml protein concentration being determined of the method of Bradford (Bradford, M., Anal. Biochem., 72, 248 (1976)). Resuspended membranes are then stored frozen at -80 °C until use.
- [0078] Binding Assay: Frozen membranes prepared as above are thawed and diluted to 1 mg protein per ml in Buffer A above. One volume of membrane preparation is combined with 0.05 volume test compound or buffer and one volume of 3 mM H<sub>3</sub>-prostaglandin E<sub>2</sub> (87RK 431, Amersham, Arlington Heights, IL) in Buffer A. The mixture (205 μL total volume) is incubated for 1 hour at 25 °C. The membranes are then recovered by filtration through type GF/C glass fiber filters (#1205-401, Walle, Gethenburg, MD) using a Tomtec harvester (Model Mach 1008, Tomtec, Orange, CT). The membranes with bound H<sub>3</sub>-prostaglandin E<sub>2</sub> are stripped by the filter, while the buffer and unbound H<sub>3</sub>-prostaglandin E<sub>2</sub> pass through the filter into waste. Each sample is then washed 3 times with 3 ml of [50 mM Tris-HCl (pH 7.4), 10 mM MgCl<sub>2</sub>, 1 mM EDTA]. The filters are then dried by heating in a microwave oven. To determine the amount of H<sub>3</sub>-prostaglandin bound to the membranes, the dried filters are placed into plastic bags with scintillation fluid and counted in a LKB 1205 Betaplate reader (Wallac, Gethenburg, MD). IC50s are determined from the concentration of test compound required to displace 50% of the specifically bound H<sub>3</sub>-prostaglandin E<sub>2</sub>.
- [0079] The full length EP<sub>1</sub> receptor is made as disclosed in Funk et al., *Journal of Biological Chemistry*, 1993, 268, 28767-28772. The full length EP<sub>2</sub> receptor is made as disclosed in Regan et al., *Molecular Pharmacology*, 1994, 46, 213-220. The full length EP<sub>3</sub> receptor is made as disclosed in Regan et al., *British Journal of Pharmacology*, 1994, 112, 327-335. The full length EP<sub>4</sub> receptor is made as disclosed in Basille, *Journal of Biological Chemistry*, 1994, 269, 11873-11877. These full length receptors are used to prepare 283S cells expressing the EP<sub>1</sub>, EP<sub>2</sub>, EP<sub>3</sub> and EP<sub>4</sub> receptors.
- [0080] 283S cells expressing either the human EP<sub>1</sub>, EP<sub>2</sub>, EP<sub>3</sub> or EP<sub>4</sub> prostaglandin E<sub>2</sub> receptors are generated according to methods known to those skilled in the art. Typically, PCR (polymerase chain reaction) primers corresponding to the 5' and 3' ends of the published full length receptor are made according to the well known methods disclosed above and are used in an RT-PCR reaction using the total RNA from human kidney (for EP<sub>1</sub>), human lung (for EP<sub>2</sub>), human lung (for EP<sub>3</sub>) or human lymphocytes (for EP<sub>4</sub>) as a source. PCR products are cloned by the TA overhang method into pCR3.1 (Invitrogen, Carlsbad, CA) and identity of the cloned receptor is confirmed by DNA sequencing. [0081] 283S cells (Mayo Dept. of Biochemistry, Northwestern Univ.) are transfected with the cloned receptor in pCDNA3 by electroporation. Stable cell lines expressing the receptor are established following selection of transfected cells with G418.

[0082] Clonal cell lines expressing the maximal number of receptors are chosen following a whole cell H<sub>3</sub>-PGE<sub>2</sub> binding assay using unlabeled PGE<sub>2</sub> as a competitor.

#### FRACTURE HEALING ASSAYS ASSAY FOR EFFECTS ON FRACTURE HEALING AFTER SYSTEMIC ADMINISTRATION

- [0083] Fracture technique: Sprague-Dawley rats at 3 months of age are anesthetized with ketamine. A 1 cm incision is made on the anteromedial aspect of the proximal part of the right tibia or femur. The following describes the tibial surgical technique. The incision is carried through to the bone, and a 1 mm hole is drilled 4 mm proximal to the distal aspect of the tibial tuberosity 2 mm medial to the anterior ridge. Intramedullary nailing is performed with a 0.8 mm stainless steel tube (maximum load 36.3 N, maximum stiffness 61.8 N/mm, tested under the same conditions as the bone). No reaming of the medullary canal is performed. A standardized closed fracture is produced 2 mm above the tibiofibular junction by three-point bending using specially designed adjustable forceps with blunt jaws. To minimize soft tissue damage, care is taken not to displace the fracture. The skin is closed with monofilament nylon sutures. The operation is performed under sterile conditions. Radiographs of all fractures are taken immediately after nailing, and rats with fractures outside the specified diaphyseal area or with displaced nails are excluded. The remaining animals are divided randomly into the following groups with 10 - 12 animals per each subgroup per time point for testing the fracture healing. The first group receives daily gavage of vehicle (water, 100% Ethanol = 65 : 5) at 1 ml/rat, while the others receive daily gavage from 0.01 to 100 mg/kg/day of the compound to be tested (1 ml/rat) for 10, 20, 40 and 80 days.
- [0084] At 10, 20, 40 and 80 days, 10 - 12 rats from each group are anesthetized with Ketamine and sacrificed by exsanguination. Both tibiofibular bones are removed by dissection and all soft tissue is stripped. Bones from 5 - 8 rats for each group are scored in 70% ethanol for histological analysis, and bones from another 5 - 6 rats for each group are stored in a buffered fluoride solution (44°C, pH 7.4) for radiographs and biomechanical testing which is performed.
- [0085] Histological Analysis: The method for histologic analysis of fractured bone have been previously published by Moseley and Bak (The Effects of Growth Hormone on Fracture Healing in Rats: A Histological Description. Bone, 14:18-27, 1993). Briefly, the fracture site is sawed 8 mm to each side of the fracture line, embedded undecalcified in methymethacrylate, and cut frontal sections on a Reichert-Jung Polycut microtome 8 μm thick. Masson-Trichrome stained mid-facial sections (including both tibia and fibula) are used for visualization of the callus and tissue response to fracture healing with and without treatment. Stains and stained sections are used to demonstrate the characteristics of the callus structure and to differentiate between woven bone and lamellar bone at the fracture site. The following measurements are performed: (1) fracture gap - measured as the shortest distance between the cortical bone ends in the fracture, (2) callus length and callus diameter, (3) total bone volume area of callus, (4) bony tissue per tissue area inside the callus area, (5) fibrous tissue in the callus, and (6) cartilage area in the callus.
- [0086] Biomechanical Analysis: The methods for biomechanical analysis have been previously published by Bak and Andreasen (The Effects of Aging on Fracture Healing in Rats. Calcif. Tissue Int. 45:282-287, 1989). Briefly, radiographs of all fractures are taken prior to the biomechanical test. The mechanical properties of the healing fractures are analyzed by a destructive three- or four-point bending procedure. Maximum load, stiffness, energy at maximum load, deflection at maximum load and maximum stress are determined.

#### ASSAY FOR EFFECTS ON FRACTURE HEALING AFTER LOCAL ADMINISTRATION

- [0087] Fracture Technique: Female or male beagle dogs at approximately 2 years of age are used under anesthesia in the study. Transverse radial fractures are produced by slow continuous loading in three-point bending as described by Lenehan et al. (Lenehan, T. M., Balligand, M., Nunamaker, D. M., Wood, F.E.: Effects of EHPD on Fracture Healing in Dogs. J Orthop Res 3:493-507, 1985). The wire is pulled through the fracture site to ensure complete anatomical disruption of the bone. Thereafter, local delivery of prostaglandin agonists to the fracture site is achieved by slow release of compound delivered by slow release pellets or by administration of the compounds in a suitable formulation such as a paste gel solution or suspension for 10, 15, or 20 weeks.
- [0088] Histological Analysis: The methods for histologic analysis of fractured bone have been previously published by Peter et al. (Peter, C.P., Cook, W.O., Nunamaker, D.M., Provost, M. T., Seedor, J.G., Rodan, G.A. Effects of alendronate on fracture healing and bone remodeling in dogs. J. Orthop. Res. 14:774-780, 1996) and Moseley and Bak (The Effects of Growth Hormone on Fracture Healing in Rats: A Histological Description. Bone, 14:18-27, 1993). Briefly, after sacrifice, the fracture site is sawed 3 cm to each side of the fracture line, embedded undecalcified in methymethacrylate, and cut on a Reichert-Jung Polycut microtome in 8 μm thick frontal sections. Masson-Trichrome stained mid-facial sections (including both tibia and fibula) are used for visualization of the callus and tissue response to fracture healing with and without treatment. Stains and stained sections are used to demonstrate the characteristics of the callus structure and to differentiate between woven bone and lamellar bone at the fracture site. The following measurements

are performed: (1) fracture gap - measured as the shortest distance between the cortical bone ends in the fracture, (2) callus length and callus diameter, (3) total bone volume area of callus, (4) bony tissue per tissue area inside the callus area, (5) fibrous tissue in the callus, (6) cartilage area in the callus.

[0098] **Biomechanical Analysis:** The methods for biomechanical analysis have been previously published by Bak and Andressen (The Effects of Aging on Fracture Healing in Rats. Calcif Tissue Int 45:292-297, 1989) and Peter et al. (Paler, C.P.; Cook, W.C.; Nunemaker, D.M.; Provost, M.T.; Seedor, G.A. Effects of Alendronate On Fracture Healing And Bone Remodeling in Dogs. J. Orthop. Res. 14:74-70, 1998). Briefly, radiographs of all fractures are taken prior to the biomechanical test. The mechanical properties of the healing fractures are analyzed by a destructive three- or four-point bending procedure. Maximum load, stiffness, energy at maximum load, deflection at maximum load, and maximum stress are determined.

#### COMBINATION AND SEQUENTIAL TREATMENT PROTOCOL

[0099] The following protocols can of course be varied by those skilled in the art. For example, intact male or female rats, sex hormone deficient male (orchidectomy) or female (ovariectomy) rats may be used. In addition, male or female rats at different ages (such as 12 months of age) can be used in the studies. The rats can be either intact or castrated (ovariectomized or orchidectomized), and administered to with anabolic agents such as the compounds of this invention at different doses (such as 1, 3 or 6 mg/kg/day) for a certain period (such as two weeks to two months), and followed by administration of an anti-resorptive agent such as zoledronic acid at different doses (such as 1.5, 10 mg/kg/day) for a certain period (such as two weeks to two months), or a combination treatment with both anabolic agent and anti-resorptive agent at different doses for a certain period (such as two weeks to two months). In the castrated rats, treatment can be started on the next day after surgery (for the purpose of preventing bone loss) or at the time bone loss has already occurred (for the purpose of restoring bone mass).

[0091] The rats are sacrificed under ketamine anesthesia. The following endpoints are determined:

[0092] Femoral bone mineral measurements are performed as described above in the estrogen agonist/antagonist protocol.

[0093] **Lumbar Vertebral Bone Mineral Measurements:** Dual energy X-ray absorptiometry (QDR 1000W, Hologic, Inc., Waltham, MA) equipped with a "Regional High Resolution Scan" software (Hologic, Inc., Waltham, MA) is used to determine the bone area, bone mineral content (BMC), and bone mineral density (BMD) of whole lumbar spine and each of the six lumbar vertebrae (L1 - 6) in the anesthetized rats. The rats are anesthetized by injection (i.p.) of 1 ml/kg of a mixture of ketamine/xylazine (ratio of 4 to 3), and then placed on a rat platform. The scan field sized is 6 x 1.9 cm, resolution is 0.0254 x 0.0127 cm, and scan speed is 7.25 mm/sec. The whole lumbar spine scan image is obtained and analyzed. Bone area (BA), and bone mineral content (BMC) is determined, and bone mineral density is calculated (BMC divided by BA) for the whole lumbar spine and each of the six lumbar vertebrae (L1 - 6).

[0094] Proximal tibial metaphyseal cancellous bone histomorphometric analyses are performed as described above for in the estrogen agonist/antagonist protocol.

[0095] Measurements and calculations related to trabecular bone volume and structure are performed as described above in the estrogen agonist/antagonist protocol. Further, measurements and calculations related to bone resorption are also performed as described above in the estrogen agonist/antagonist protocol. Still further, measurements and calculations related to bone formation and turnover are performed as described above in the estrogen agonist/antagonist protocol. Further still, the data obtained is analyzed using the statistical manipulations described above in the estrogen agonist/antagonist protocol.

[0096] Administration of the compositions of this invention or of a combination of an  $EP_2$  receptor selective agonist, a prodrug thereof or a pharmaceutically acceptable salt of said agonist or said prodrug and an  $EP_4$  receptor selective agonist, a prodrug thereof or a pharmaceutically acceptable salt of said agonist or said prodrug can be via any method which delivers the composition of this invention or  $EP_2$  receptor selective agonist and  $EP_4$  receptor selective agonist systemically and/or locally (e.g., at the site of the bone fracture, osteotomy, or orthopedic surgery). These methods include oral routes, parenteral, intraduodenal routes, etc. Generally, the compounds of this invention are administered orally, but parenteral administration (e.g., intravenous, intramuscular, transdermal, subcutaneous, rectal or intramedullary) may be utilized, for example, where oral administration is inappropriate for the target or where the patient is unable to ingest the drug.

[0097] The pharmaceutical compositions of this invention and the  $EP_2$  receptor selective agonist and  $EP_4$  receptor selective agonist combinations can be used for the treatment and promotion of healing of bone fractures and osteotomies by the local application (e.g., to the sites of bone fractures or osteotomies) of the compositions of this invention. The compositions of this invention and the  $EP_2$  receptor selective agonist and  $EP_4$  receptor selective agonist are applied to the sites of bone fractures or osteotomies, for example, either by injection of the compound in a suitable solvent (e.g., an oily solvent such as arachis oil) to the cartilage growth plate or, in cases of open surgery, by local application thereto of such compositions in a suitable vehicle, carrier or diluent such as bone wax, demineralized bone

powder, polymeric bone cements, bone sealants, etc. Alternatively, local application can be achieved by applying a solution or dispersion of the composition or the  $EP_2$  receptor selective agonist and  $EP_4$  receptor selective agonist combination in a suitable carrier or diluent onto the surface of, or incorporating it into solid or semi-solid implants conventionally used in orthopedic surgery, such as debrion-mesh, gel-foam and ket bone, or prostheses.

[0098] In any event, the amount and timing of compositions administered will, of course, be dependent on the subject being treated, on the severity of the affliction, on the manner of administration and on the judgment of the prescribing physician. Thus, because of patient to patient variability, the dosages given above are a guideline and the physician may titrate doses of the drug compounds to achieve the treatment (e.g., bone mass augmentation) that the physician considers appropriate for the patient. In considering the degree of treatment desired, the physician must balance a variety of factors such as bone mass starting level, age of the patient, presence of preexisting disease, as well as presence of other diseases (e.g., cardiovascular disease).

[0099] In general an amount of a composition or combination of an  $EP_2$  receptor selective agonist and  $EP_4$  receptor selective agonist of this invention is used that is sufficient to augment bone mass to a level which is above the bone fracture threshold (as detailed in the World Health Organization Study previously cited herein).

[0100] The  $EP_2$  receptor selective compounds and  $EP_4$  receptor selective compounds used in the compositions, methods and kits of the present invention are generally administered in the form of a pharmaceutical composition comprising at least one of the compounds of this invention together with a pharmaceutically acceptable vehicle or diluent. Thus, the compounds of this invention can be administered individually or together in any conventional form such as oral, parenteral, rectal or transdermal dosage form.

[0101] For oral administration the pharmaceutical composition can take the form of solutions, suspensions, tablets, pills, capsules, powders, and the like. Tablets containing various excipients such as sodium citrate, calcium carbonate and calcium phosphate are employed along with various disintegrants such as starch and preferably potato or tapioca starch and certain complex silicates, together with binding agents such as polyvinylpyrrolidone, sucrose, gelatin and acacia. Additionally, lubricating agents such as magnesium stearate, sodium lauryl sulfate and talc are often very useful for tabletting purposes. Solid compositions of a similar type are also employed as fillers in soft and hard-filled gelatin capsules; preferred materials in this connection also include lactose or milk sugar as well as high molecular weight polyethylene glycols. When aqueous suspensions and/or elixirs are desired for oral administration, the compositions of this invention can be combined with various sweetening agents, flavoring agents, coloring agents, emulsifying agents and/or suspending agents, as well as such diluents as water, ethanol, propylene glycol, glycerin and various like combinations thereof.

[0102] For purposes of parenteral administration, solutions in sesame or peanut oil or in aqueous propylene glycol can be employed, as well as sterile aqueous solutions of the corresponding water-soluble salts. Such aqueous solutions may be suitably buffered. If necessary, and the liquid diluent first rendered isotonic with sufficient saline or glucose. These aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal injection purposes. In this connection, the sterile aqueous media employed are all readily obtainable by standard techniques well-known to those skilled in the art.

[0103] For purposes of transdermal (e.g., topical) administration, dilute sterile, aqueous or partially aqueous solutions (usually in about 0.1% to 5% concentration), otherwise similar to the above parenteral solutions, are prepared.

[0104] Methods of preparing various pharmaceutical compositions with a certain amount of active ingredient are known, or will be apparent in light of this disclosure, to those skilled in this art. For examples of methods of preparing pharmaceutical compositions, see, Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., 18th Edition (1985).

[0105] Pharmaceutical compositions of the invention may contain a total of 0.1%-95% of an  $EP_2$  receptor selective agonist and of an  $EP_4$  receptor selective agonist used in this invention, preferably 1%-70%. In any event, the composition or formulation to be administered will contain a quantity of the  $EP_2$  receptor selective agonist and the  $EP_4$  receptor selective agonist in an amount effective to treat the disease/condition of the subject being treated, e.g., a bone disorder. [0106] Since the present invention has an aspect that relates to the augmentation and maintenance of bone mass by treatment with a combination of active ingredients which may be administered separately, the invention also relates to combining separate pharmaceutical compositions in kit form. The kit comprises two separate pharmaceutical compositions, an  $EP_2$  receptor selective compound, a prodrug thereof or a pharmaceutically acceptable salt of said  $EP_2$  receptor selective compound or of said prodrug, and an  $EP_4$  receptor selective compound, a prodrug thereof or a pharmaceutically acceptable salt of said  $EP_4$  receptor selective compound or of said prodrug as described above. The kit comprises a container for containing the separate compositions such as a divided bottle or a divided foil packet, however, the separate compositions may also be contained within a single, undivided container. Typically the kit comprises directions for the administration of the separate components. The kit form is particularly advantageous when the separate components are preferably administered in different dosage forms (e.g., oral and parenteral), are administered at different dosage intervals, or when titration of the individual components of the combination is desired by the prescribing physician.

[0107] An example of such a kit is a so-called blister pack. Blister packs are well known in the packaging industry and are being widely used for the packaging of pharmaceutical unit dosage forms (tablets, capsules, and the like). Blister packs generally consist of a sheet of relatively stiff material covered with a foil of a preferably transparent plastic material. During the packaging process recesses are formed in the plastic foil. The recesses have the size and shape of the tablets or capsules to be packed. Next, the tablets or capsules are placed in the recesses and the sheet of relatively stiff material is sealed against the plastic foil at the face of the foil which is opposite from the direction in which the recesses were formed. As a result, the tablets or capsules are sealed in the recesses between the plastic foil and the sheet. Preferably the strength of the sheet is such that the tablets or capsules can be removed from the blister pack by manually applying pressure on the recesses whereby an opening is formed in the sheet at the place of the recess. The tablet or capsule can then be removed via said opening.

[0108] It may be desirable to provide a memory aid on the kit, e.g., in the form of numbers next to the tablets or capsules whereby the numbers correspond with the days of the regimen which the dosage form so specified should be ingested. Another example of such a memory aid is a calendar printed on the card e.g., as follows "First Week, Monday, Tuesday, ... etc. Second Week, Monday, Tuesday, ... etc. Other variations of memory aids will be readily apparent. A "daily dose" can be a single tablet or capsule or several tablets or capsules to be taken on a given day. Also, a daily dose of an EP<sub>2</sub> receptor selective compound, producing therof or pharmacologically acceptable salt of said EP<sub>2</sub> receptor selective compound or said producting can consist of one tablet or capsule while a daily dose of the EP<sub>4</sub> receptor selective compound, producing therof or pharmacologically acceptable salt of said EP<sub>4</sub> receptor selective compound or said producting can consist of several tablets or capsules and vice versa. The memory aid should reflect this.

[0109] In another specific embodiment of the invention, a dispenser designed to dispense the daily doses one at a time in the order of their intended use is provided. Preferably, the dispenser is equipped with a memory aid, so as to further facilitate compliance with the regimen. An example of such a memory-aid is a mechanical counter which indicates the number of daily doses that has been dispensed. Another example of such a memory-aid is a battery-powered micro-chip memory coupled with a liquid crystal readout, or audible reminder signal which, for example, reads out the date that the last daily dose has been taken and/or reminds one when the next dose is to be taken.

#### Example One

##### Study Protocol

[0110] Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) restores bone mass by stimulating both bone formation and bone resorption but in favor of bone formation in ovariectomized (OVX) rat skeleton. The purpose of this study was to determine the skeletal effects of 3-(3-hydroxy-4-phenyl-butyl)-2-(6-(1-H-tetrazol-5-yl)-hexyl)-cyclopentanone, an EP<sub>2</sub> and EP<sub>4</sub> prostaglandin receptor selective agonist (CS50 for EP<sub>2</sub> and EP<sub>4</sub> receptor binding equals 56 and 70 nM, respectively, [CS50 for EP<sub>1</sub>, EP<sub>3</sub>, DP and FP receptor binding are all >3200 nM] in ovariectomized OVX rats.

[0111] Sprague-Dawley female rats were sham-operated (n=20) or OVX (n=50) at 3 months of age. Five weeks post-surgery, OVX rats were treated (subcutaneous injection) with either vehicle or 3-(3-hydroxy-4-phenyl-butyl)-2-(6-(1-H-tetrazol-5-yl)-hexyl)-cyclopentanone at 10 mg/kg/day for 4 weeks. Distal femoral metaphyseal bone mineral content (BMC) and bone mineral density (BMD) were determined by dual energy x-ray absorptiometry (Hologic ODR-1000W, Hologic Inc., Waltham, MA) according to the methods described by Ke et al., (Dronizolene, a New Estrogen Antagonist/Agonist, Prevents Bone Loss in Ovariectomized Rats, Endocrinology, 136:2435-2441, 1995).

##### Study Results and Discussion

[0112] OVX induced significant decrease in BMC (-15%) and BMD (-17%) at 5 weeks post-surgery as compared with sham-operated controls. Continuous decreases in BMC and BMD were seen between 5 and 8 weeks post-surgery in OVX rats (-23% in both BMC and BMD). 3-(3-hydroxy-4-phenyl-butyl)-2-(6-(1-H-tetrazol-5-yl)-hexyl)-cyclopentanone at 10 mg/kg/day significantly increased BMC and BMD as compared with pre-treatment OVX controls (+23% and +17% for BMC and BMD, respectively) and OVX controls (-42% and +28% for BMC and BMD, respectively).

[0113] These data showed that 3-(3-hydroxy-4-phenyl-butyl)-2-(6-(1-H-tetrazol-5-yl)-hexyl)-cyclopentanone, an EP<sub>2</sub> EP<sub>4</sub> receptor selective agonist, completely restored the bone mass to the OVX, ovariectomized rat skeleton. These results indicated EP<sub>2</sub>EP<sub>4</sub> receptor selective agonists are useful agents in treatment of osteoporosis.

##### Example Two

##### Study Protocol

[0114] Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) restores bone mass by stimulating both bone formation and bone resorption but in

favor of bone formation in ovariectomized (OVX) rat skeleton. The purpose of this study was to determine the skeletal effects of combination of 7-(2-(3,5-dichloro-phenoxyl)-ethyl)-methanesulfonyl-aminol-heptanoic acid, an EP<sub>2</sub> prostaglandin receptor selective agonist (CS50 for EP<sub>2</sub> receptor binding equals 17 nM), and 7-(2-(3-hydroxy-4-phenyl-butyl)-5-oxo-pyrrolidin-1-yl)-heptanoic acid, an EP<sub>4</sub> prostaglandin receptor selective agonist (CS50 for EP<sub>4</sub> receptor binding equals 58 nM) in ovariectomized OVX rats.

[0115] Sprague-Dawley female rats were sham-operated (n=20) or OVX (n=50) at 3 months of age. Five weeks post-surgery, OVX rats were treated (subcutaneous injection) with either vehicle or combination of 7-(2-(3,5-dichloro-phenoxyl)-ethyl)-methanesulfonyl-aminol-heptanoic acid (10 mg/kg/day, s.c. injection) and 7-(2-(3-hydroxy-4-phenyl-butyl)-5-oxo-pyrrolidin-1-yl)-heptanoic acid (10 mg/kg/day, s.c. injection) for 4 weeks. Distal femoral metaphyseal bone mineral content (BMC) and bone mineral density (BMD) were determined by dual energy x-ray absorptiometry (Hologic ODR-1000W, Hologic Inc., Waltham, MA) according to the methods described by Ke et al., (Dronizolene, a New Estrogen Antagonist/Agonist, Prevents Bone Loss in Ovariectomized Rats, Endocrinology, 136:2435-2441, 1995).

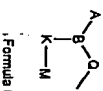
##### Study Results and Discussion

[0116] OVX induced significant decrease in BMC (-13%) and BMD (-13%) at 5 weeks post-surgery as compared with sham-operated controls. Continuous decreases in BMC and BMD were seen between 5 and 8 weeks post-surgery in OVX rats (-26% in BMC and -24% in BMD). A combination of 7-(2-(3,5-dichloro-phenoxyl)-ethyl)-methanesulfonyl-aminol-heptanoic acid (10 mg/kg/day, s.c. injection) and 7-(2-(3-hydroxy-4-phenyl-butyl)-5-oxo-pyrrolidin-1-yl)-heptanoic acid (10 mg/kg/day, s.c. injection) significantly increased BMC and BMD as compared with pre-treatment OVX controls (+14% and +8% for BMC and BMD, respectively) and OVX controls (-416% and +12% for BMC and BMD, respectively).

[0117] These data show that treatment with combination of an EP<sub>2</sub> and an EP<sub>4</sub> receptor selective agonists restored bone mass to the OVX, ovariectomized rat skeleton. These results demonstrate that these therapeutic regimens are useful in treatment of osteoporosis.

##### Claims

1. The use of an EP<sub>2</sub> receptor selective agonist, producing or pharmacologically acceptable salt thereof in the preparation of a medicament in combination with an EP<sub>4</sub> receptor selective agonist, producing or a pharmacologically acceptable salt thereof, for the treatment of a condition which presents with low bone mass in mammals.
2. The use as claimed in claim 1 wherein said EP<sub>2</sub> receptor selective agonist is selected from:



Formula I

producing therof, and the pharmaceutically acceptable salts of the compounds and the products, wherein:

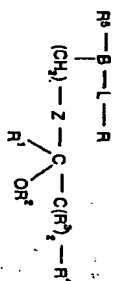
- B is N;  
A is (C<sub>1</sub>-C<sub>6</sub>)alkylsulfonyl, (C<sub>3</sub>-C<sub>7</sub>)cycloalkylsulfonyl, (C<sub>3</sub>-C<sub>7</sub>)cycloalkylsulfonyl, said A moieties optionally mono-, di- or tri- substituted on carbon independently with hydroxy, (C<sub>1</sub>-C<sub>6</sub>)alkyl or halo;  
O is  
- (C<sub>2</sub>-C<sub>6</sub>)alkylene-W-(C<sub>1</sub>-C<sub>6</sub>)alkylene,  
- (C<sub>3</sub>-C<sub>7</sub>)alkylene- said (C<sub>3</sub>-C<sub>7</sub>)alkylene- optionally substituted with up to four substituents independently selected from fluoro or (C<sub>1</sub>-C<sub>6</sub>)alkyl,  
-X-(C<sub>1</sub>-C<sub>6</sub>)alkylene-  
- (C<sub>1</sub>-C<sub>6</sub>)alkylene-X-  
- (C<sub>1</sub>-C<sub>6</sub>)alkylene-X-(C<sub>1</sub>-C<sub>6</sub>)alkylene-  
- (C<sub>2</sub>-C<sub>6</sub>)alkylene-W-X-(C<sub>1</sub>-C<sub>6</sub>)alkylene-  
- (C<sub>3</sub>-C<sub>7</sub>)alkylene-X-W-(C<sub>1</sub>-C<sub>6</sub>)alkylene-  
- (C<sub>2</sub>-C<sub>6</sub>)alkylene-W-X-W-(C<sub>1</sub>-C<sub>6</sub>)alkylene-, wherein the two occurrences of W are independent of each other.



[illegible]

W is oxy, thio, sulfinio, sulfinyl, amino, sulfonyl, *n*-mono-N-(C-*C*<sub>1</sub>-C<sub>2</sub>-C<sub>3</sub>-C<sub>4</sub>-C<sub>5</sub>-C<sub>6</sub>-C<sub>7</sub>-C<sub>8</sub>-C<sub>9</sub>-C<sub>10</sub>-C<sub>11</sub>-C<sub>12</sub>-C<sub>13</sub>-C<sub>14</sub>-C<sub>15</sub>-C<sub>16</sub>-C<sub>17</sub>-C<sub>18</sub>-C<sub>19</sub>-C<sub>20</sub>-C<sub>21</sub>-C<sub>22</sub>-C<sub>23</sub>-C<sub>24</sub>-C<sub>25</sub>-C<sub>26</sub>-C<sub>27</sub>-C<sub>28</sub>-C<sub>29</sub>-C<sub>30</sub>-C<sub>31</sub>-C<sub>32</sub>-C<sub>33</sub>-C<sub>34</sub>-C<sub>35</sub>-C<sub>36</sub>-C<sub>37</sub>-C<sub>38</sub>-C<sub>39</sub>-C<sub>40</sub>-C<sub>41</sub>-C<sub>42</sub>-C<sub>43</sub>-C<sub>44</sub>-C<sub>45</sub>-C<sub>46</sub>-C<sub>47</sub>-C<sub>48</sub>-C<sub>49</sub>-C<sub>50</sub>-C<sub>51</sub>-C<sub>52</sub>-C<sub>53</sub>-C<sub>54</sub>-C<sub>55</sub>-C<sub>56</sub>-C<sub>57</sub>-C<sub>58</sub>-C<sub>59</sub>-C<sub>60</sub>-C<sub>61</sub>-C<sub>62</sub>-C<sub>63</sub>-C<sub>64</sub>-C<sub>65</sub>-C<sub>66</sub>-C<sub>67</sub>-C<sub>68</sub>-C<sub>69</sub>-C<sub>70</sub>-C<sub>71</sub>-C<sub>72</sub>-C<sub>73</sub>-C<sub>74</sub>-C<sub>75</sub>-C<sub>76</sub>-C<sub>77</sub>-C<sub>78</sub>-C<sub>79</sub>-C<sub>80</sub>-C<sub>81</sub>-C<sub>82</sub>-C<sub>83</sub>-C<sub>84</sub>-C<sub>85</sub>-C<sub>86</sub>-C<sub>87</sub>-C<sub>88</sub>-C<sub>89</sub>-C<sub>90</sub>-C<sub>91</sub>-C<sub>92</sub>-C<sub>93</sub>-C<sub>94</sub>-C<sub>95</sub>-C<sub>96</sub>-C<sub>97</sub>-C<sub>98</sub>-C<sub>99</sub>-C<sub>100</sub>-C<sub>101</sub>-C<sub>102</sub>-C<sub>103</sub>-C<sub>104</sub>-C<sub>105</sub>-C<sub>106</sub>-C<sub>107</sub>-C<sub>108</sub>-C<sub>109</sub>-C<sub>110</sub>-C<sub>111</sub>-C<sub>112</sub>-C<sub>113</sub>-C<sub>114</sub>-C<sub>115</sub>-C<sub>116</sub>-C<sub>117</sub>-C<sub>118</sub>-C<sub>119</sub>-C<sub>120</sub>-C<sub>121</sub>-C<sub>122</sub>-C<sub>123</sub>-C<sub>124</sub>-C<sub>125</sub>-C<sub>126</sub>-C<sub>127</sub>-C<sub>128</sub>-C<sub>129</sub>-C<sub>130</sub>-C<sub>131</sub>-C<sub>132</sub>-C<sub>133</sub>-C<sub>134</sub>-C<sub>135</sub>-C<sub>136</sub>-C<sub>137</sub>-C<sub>138</sub>-C<sub>139</sub>-C<sub>140</sub>-C<sub>141</sub>-C<sub>142</sub>-C<sub>143</sub>-C<sub>144</sub>-C<sub>145</sub>-C<sub>146</sub>-C<sub>147</sub>-C<sub>148</sub>-C<sub>149</sub>-C<sub>150</sub>-C<sub>151</sub>-C<sub>152</sub>-C<sub>153</sub>-C<sub>154</sub>-C<sub>155</sub>-C<sub>156</sub>-C<sub>157</sub>-C<sub>158</sub>-C<sub>159</sub>-C<sub>160</sub>-C<sub>161</sub>-C<sub>162</sub>-C<sub>163</sub>-C<sub>164</sub>-C<sub>165</sub>-C<sub>166</sub>-C<sub>167</sub>-C<sub>168</sub>-C<sub>169</sub>-C<sub>170</sub>-C<sub>171</sub>-C<sub>172</sub>-C<sub>173</sub>-C<sub>174</sub>-C<sub>175</sub>-C<sub>176</sub>-C<sub>177</sub>-C<sub>178</sub>-C<sub>179</sub>-C<sub>180</sub>-C<sub>181</sub>-C<sub>182</sub>-C<sub>183</sub>-C<sub>184</sub>-C<sub>185</sub>-C<sub>186</sub>-C<sub>187</sub>-C<sub>188</sub>-C<sub>189</sub>-C<sub>190</sub>-C<sub>191</sub>-C<sub>192</sub>-C<sub>193</sub>-C<sub>194</sub>-C<sub>195</sub>-C<sub>196</sub>-C<sub>197</sub>-C<sub>198</sub>-C<sub>199</sub>-C<sub>200</sub>-C<sub>201</sub>-C<sub>202</sub>-C<sub>203</sub>-C<sub>204</sub>-C<sub>205</sub>-C<sub>206</sub>-C<sub>207</sub>-C<sub>208</sub>-C<sub>209</sub>-C<sub>210</sub>-C<sub>211</sub>-C<sub>212</sub>-C<sub>213</sub>-C<sub>214</sub>-C<sub>215</sub>-C<sub>216</sub>-C<sub>217</sub>-C<sub>218</sub>-C<sub>219</sub>-C<sub>220</sub>-C<sub>221</sub>-C<sub>222</sub>-C<sub>223</sub>-C<sub>224</sub>-C<sub>225</sub>-C<sub>226</sub>-C<sub>227</sub>-C<sub>228</sub>-C<sub>229</sub>-C<sub>230</sub>-C<sub>231</sub>-C<sub>232</sub>-C<sub>233</sub>-C<sub>234</sub>-C<sub>235</sub>-C<sub>236</sub>-C<sub>237</sub>-C<sub>238</sub>-C<sub>239</sub>-C<sub>240</sub>-C<sub>241</sub>-C<sub>242</sub>-C<sub>243</sub>-C<sub>244</sub>-C<sub>245</sub>-C<sub>246</sub>-C<sub>247</sub>-C<sub>248</sub>-C<sub>249</sub>-C<sub>250</sub>-C<sub>251</sub>-C<sub>252</sub>-C<sub>253</sub>-C<sub>254</sub>-C<sub>255</sub>-C<sub>256</sub>-C<sub>257</sub>-C<sub>258</sub>-C<sub>259</sub>-C<sub>260</sub>-C<sub>261</sub>-C<sub>262</sub>-C<sub>263</sub>-C<sub>264</sub>-C<sub>265</sub>-C<sub>266</sub>-C<sub>267</sub>-C<sub>268</sub>-C<sub>269</sub>-C<sub>270</sub>-C<sub>271</sub>-C<sub>272</sub>-C<sub>273</sub>-C<sub>274</sub>-C<sub>275</sub>-C<sub>276</sub>-C<sub>277</sub>-C<sub>278</sub>-C<sub>279</sub>-C<sub>280</sub>-C<sub>281</sub>-C<sub>282</sub>-C<sub>283</sub>-C<sub>284</sub>-C<sub>285</sub>-C<sub>286</sub>-C<sub>287</sub>-C<sub>288</sub>-C<sub>289</sub>-C<sub>290</sub>-C<sub>291</sub>-C<sub>292</sub>-C<sub>293</sub>-C<sub>294</sub>-C<sub>295</sub>-C<sub>296</sub>-C<sub>297</sub>-C<sub>298</sub>-C<sub>299</sub>-C<sub>300</sub>-C<sub>301</sub>-C<sub>302</sub>-C<sub>303</sub>-C<sub>304</sub>-C<sub>305</sub>-C<sub>306</sub>-C<sub>307</sub>-C<sub>308</sub>-C<sub>309</sub>-C<sub>310</sub>-C<sub>311</sub>-C<sub>312</sub>-C<sub>313</sub>-C<sub>314</sub>-C<sub>315</sub>-C<sub>316</sub>-C<sub>317</sub>-C<sub>318</sub>-C<sub>319</sub>-C<sub>320</sub>-C<sub>321</sub>-C<sub>322</sub>-C<sub>323</sub>-C<sub>324</sub>-C<sub>325</sub>-C<sub>326</sub>-C<sub>327</sub>-C<sub>328</sub>-C<sub>329</sub>-C<sub>330</sub>-C<sub>331</sub>-C<sub>332</sub>-C<sub>333</sub>-C<sub>334</sub>-C<sub>335</sub>-C<sub>336</sub>-C<sub>337</sub>-C<sub>338</sub>-C<sub>339</sub>-C<sub>340</sub>-C<sub>341</sub>-C<sub>342</sub>-C<sub>343</sub>-C<sub>344</sub>-C<sub>345</sub>-C<sub>346</sub>-C<sub>347</sub>-C<sub>348</sub>-C<sub>349</sub>-C<sub>350</sub>-C<sub>351</sub>-C<sub>352</sub>-C<sub>353</sub>-C<sub>354</sub>-C<sub>355</sub>-C<sub>356</sub>-C<sub>357</sub>-C<sub>358</sub>-C<sub>359</sub>-C<sub>360</sub>-C<sub>361</sub>-C<sub>362</sub>-C<sub>363</sub>-C<sub>364</sub>-C<sub>365</sub>-C<sub>366</sub>-C<sub>367</sub>-C<sub>368</sub>-C<sub>369</sub>-C<sub>370</sub>-C<sub>371</sub>-C<sub>372</sub>-C<sub>373</sub>-C<sub>374</sub>-C<sub>375</sub>-C<sub>376</sub>-C<sub>377</sub>-C<sub>378</sub>-C<sub>379</sub>-C<sub>380</sub>-C<sub>381</sub>-C<sub>382</sub>-C<sub>383</sub>-C<sub>384</sub>-C<sub>385</sub>-C<sub>386</sub>-C<sub>387</sub>-C<sub>388</sub>-C<sub>389</sub>-C<sub>390</sub>-C<sub>391</sub>-C<sub>392</sub>-C<sub>393</sub>-C<sub>394</sub>-C<sub>395</sub>-C<sub>396</sub>-C<sub>397</sub>-C<sub>398</sub>-C<sub>399</sub>-C<sub>400</sub>-C<sub>401</sub>-C<sub>402</sub>-C<sub>403</sub>-C<sub>404</sub>-C<sub>405</sub>-C<sub>406</sub>-C<sub>407</sub>-C<sub>408</sub>-C<sub>409</sub>-C<sub>410</sub>-C<sub>411</sub>-C<sub>412</sub>-C<sub>413</sub>-C<sub>414</sub>-C<sub>415</sub>-C<sub>416</sub>-C<sub>41</sub>

X is a *div*- or *disubstituted aromatic ring* optionally having one or two heteroatoms selected independently with halide from oxygen, nitrogen, and sulfur; said ring optionally mono-, di- or tri-substituted independently with halide from (C<sub>1</sub>-C<sub>3</sub>alkyl), fluoromethyl, trifluoromethyl, difluoromethyl, hydroxyl, (C<sub>1</sub>-C<sub>3</sub>alkoxy), or carbamoyl; R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, R<sup>8</sup>, R<sup>9</sup>, R<sup>10</sup>, R<sup>11</sup>, R<sup>12</sup>, R<sup>13</sup>, R<sup>14</sup>, R<sup>15</sup>, R<sup>16</sup>, R<sup>17</sup>, R<sup>18</sup>, R<sup>19</sup>, R<sup>20</sup>, R<sup>21</sup>, R<sup>22</sup>, R<sup>23</sup>, R<sup>24</sup>, R<sup>25</sup>, R<sup>26</sup>, R<sup>27</sup>, R<sup>28</sup>, R<sup>29</sup>, R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup>, R<sup>34</sup>, R<sup>35</sup>, R<sup>36</sup>, R<sup>37</sup>, R<sup>38</sup>, R<sup>39</sup>, R<sup>40</sup>, R<sup>41</sup>, R<sup>42</sup>, R<sup>43</sup>, R<sup>44</sup>, R<sup>45</sup>, R<sup>46</sup>, R<sup>47</sup>, R<sup>48</sup>, R<sup>49</sup>, R<sup>50</sup>, R<sup>51</sup>, R<sup>52</sup>, R<sup>53</sup>, R<sup>54</sup>, R<sup>55</sup>, R<sup>56</sup>, R<sup>57</sup>, R<sup>58</sup>, R<sup>59</sup>, R<sup>60</sup>, R<sup>61</sup>, R<sup>62</sup>, R<sup>63</sup>, R<sup>64</sup>, R<sup>65</sup>, R<sup>66</sup>, R<sup>67</sup>, R<sup>68</sup>, R<sup>69</sup>, R<sup>70</sup>, R<sup>71</sup>, R<sup>72</sup>, R<sup>73</sup>, R<sup>74</sup>, R<sup>75</sup>, R<sup>76</sup>, R<sup>77</sup>, R<sup>78</sup>, R<sup>79</sup>, R<sup>80</sup>, R<sup>81</sup>, R<sup>82</sup>, R<sup>83</sup>, R<sup>84</sup>, R<sup>85</sup>, R<sup>86</sup>, R<sup>87</sup>, R<sup>88</sup>, R<sup>89</sup>, R<sup>90</sup>, R<sup>91</sup>, R<sup>92</sup>, R<sup>93</sup>, R<sup>94</sup>, R<sup>95</sup>, R<sup>96</sup>, R<sup>97</sup>, R<sup>98</sup>, R<sup>99</sup>, R<sup>100</sup>, R<sup>101</sup>, R<sup>102</sup>, R<sup>103</sup>, R<sup>104</sup>, R<sup>105</sup>, R<sup>106</sup>, R<sup>107</sup>, R<sup>108</sup>, R<sup>109</sup>, R<sup>110</sup>, R<sup>111</sup>, R<sup>112</sup>, R<sup>113</sup>, R<sup>114</sup>, R<sup>115</sup>, R<sup>116</sup>, R<sup>117</sup>, R<sup>118</sup>, R<sup>119</sup>, R<sup>120</sup>, R<sup>121</sup>, R<sup>122</sup>, R<sup>123</sup>, R<sup>124</sup>, R<sup>125</sup>, R<sup>126</sup>, R<sup>127</sup>, R<sup>128</sup>, R<sup>129</sup>, R<sup>130</sup>, R<sup>131</sup>, R<sup>132</sup>, R<sup>133</sup>, R<sup>134</sup>, R<sup>135</sup>, R<sup>136</sup>, R<sup>137</sup>, R<sup>138</sup>, R<sup>139</sup>, R<sup>140</sup>, R<sup>141</sup>, R<sup>142</sup>, R<sup>143</sup>, R<sup>144</sup>, R<sup>145</sup>, R<sup>146</sup>, R<sup>147</sup>, R<sup>148</sup>, R<sup>149</sup>, R<sup>150</sup>, R<sup>151</sup>, R<sup>152</sup>, R<sup>153</sup>, R<sup>154</sup>, R<sup>155</sup>, R<sup>156</sup>, R<sup>157</sup>, R<sup>158</sup>, R<sup>159</sup>, R<sup>160</sup>, R<sup>161</sup>, R<sup>162</sup>, R<sup>163</sup>, R<sup>164</sup>, R<sup>165</sup>, R<sup>166</sup>, R<sup>167</sup>, R<sup>168</sup>, R<sup>169</sup>, R<sup>170</sup>, R<sup>171</sup>, R<sup>172</sup>, R<sup>173</sup>, R<sup>174</sup>, R<sup>175</sup>, R<sup>176</sup>, R<sup>177</sup>, R<sup>178</sup>, R<sup>179</sup>, R<sup>180</sup>, R<sup>181</sup>, R<sup>182</sup>, R<sup>183</sup>, R<sup>184</sup>, R<sup>185</sup>, R<sup>186</sup>, R<sup>187</sup>, R<sup>188</sup>, R<sup>189</sup>, R<sup>190</sup>, R<sup>191</sup>, R<sup>192</sup>, R<sup>193</sup>, R<sup>194</sup>, R<sup>195</sup>, R<sup>196</sup>, R<sup>197</sup>, R<sup>198</sup>, R<sup>199</sup>, R<sup>200</sup>, R<sup>201</sup>, R<sup>202</sup>, R<sup>203</sup>, R<sup>204</sup>, R<sup>205</sup>, R<sup>206</sup>, R<sup>207</sup>, R<sup>208</sup>, R<sup>209</sup>, R<sup>210</sup>, R<sup>211</sup>, R<sup>212</sup>, R<sup>213</sup>, R<sup>214</sup>, R<sup>215</sup>, R<sup>216</sup>, R<sup>217</sup>, R<sup>218</sup>, R<sup>219</sup>, R<sup>220</sup>, R<sup>221</sup>, R<sup>222</sup>, R<sup>223</sup>, R<sup>224</sup>, R<sup>225</sup>, R<sup>226</sup>, R<sup>227</sup>, R<sup>228</sup>, R<sup>229</sup>, R<sup>230</sup>, R<sup>231</sup>, R<sup>232</sup>, R<sup>233</sup>, R<sup>234</sup>, R<sup>235</sup>, R<sup>236</sup>, R<sup>237</sup>, R<sup>238</sup>, R<sup>239</sup>, R<sup>240</sup>, R<sup>241</sup>, R<sup>242</sup>, R<sup>243</sup>, R<sup>244</sup>, R<sup>245</sup>, R<sup>246</sup>, R<sup>247</sup>, R<sup>248</sup>, R<sup>249</sup>, R<sup>250</sup>, R<sup>251</sup>, R<sup>252</sup>, R<sup>253</sup>, R<sup>254</sup>, R<sup>255</sup>, R<sup>256</sup>, R<sup>257</sup>, R<sup>258</sup>, R<sup>259</sup>, R<sup>260</sup>, R<sup>261</sup>, R<sup>262</sup>, R<sup>263</sup>, R<sup>264</sup>, R<sup>265</sup>, R<sup>266</sup>, R<sup>267</sup>, R<sup>268</sup>, R<sup>269</sup>, R<sup>270</sup>, R<sup>271</sup>, R<sup>272</sup>, R<sup>273</sup>, R<sup>274</sup>, R<sup>275</sup>, R<sup>276</sup>, R<sup>277</sup>, R<sup>278</sup>, R<sup>279</sup>, R<sup>280</sup>, R<sup>281</sup>, R<sup>282</sup>, R<sup>283</sup>, R<sup>284</sup>, R<sup>285</sup>, R<sup>286</sup>, R<sup>287</sup>, R<sup>288</sup>, R<sup>289</sup>, R<sup>290</sup>, R<sup>291</sup>, R<sup>292</sup>, R<sup>293</sup>, R<sup>294</sup>, R<sup>295</sup>, R<sup>296</sup>, R<sup>297</sup>, R<sup>298</sup>, R<sup>299</sup>, R<sup>300</sup>, R<sup>301</sup>, R<sup>302</sup>, R<sup>303</sup>, R<sup>304</sup>, R<sup>305</sup>, R<sup>306</sup>, R<sup>307</sup>, R<sup>308</sup>, R<sup>309</sup>, R<sup>310</sup>, R<sup>311</sup>, R<sup>312</sup>, R<sup>313</sup>, R<sup>314</sup>, R<sup>315</sup>, R<sup>316</sup>, R<sup>317</sup>, R<sup>318</sup>, R<sup>319</sup>, R<sup>320</sup>, R<sup>321</sup>, R<sup>322</sup>, R<sup>323</sup>, R<sup>324</sup>, R<sup>325</sup>, R<sup>326</sup>, R<sup>327</sup>, R<sup>328</sup>, R<sup>329</sup>, R<sup>330</sup>, R<sup>331</sup>, R<sup>332</sup>, R<sup>333</sup>, R<sup>334</sup>, R<sup>335</sup>, R<sup>336</sup>, R<sup>337</sup>, R<sup>338</sup>, R<sup>339</sup>, R<sup>340</sup>, R<sup>341</sup>, R<sup>342</sup>, R<sup>343</sup>, R<sup>344</sup>, R<sup>345</sup>, R<sup>346</sup>, R<sup>347</sup>, R<sup>348</sup>, R<sup>349</sup>, R<sup>350</sup>, R<sup>351</sup>, R<sup>352</sup>, R<sup>353</sup>, R<sup>354</sup>, R<sup>355</sup>, R<sup>356</sup>, R<sup>357</sup>, R<sup>358</sup>, R<sup>359</sup>, R<sup>360</sup>, R<sup>361</sup>, R<sup>362</sup>, R<sup>363</sup>, R<sup>364</sup>, R<sup>365</sup>, R<sup>366</sup>, R<sup>367</sup>, R<sup>368</sup>, R<sup>369</sup>, R<sup>370</sup>, R<sup>371</sup>, R<sup>372</sup>, R<sup>373</sup>, R<sup>374</sup>, R<sup>375</sup>, R<sup>376</sup>, R<sup>377</sup>, R<sup>378</sup>, R<sup>379</sup>, R<sup>380</sup>, R<sup>381</sup>, R<sup>382</sup>, R<sup>383</sup>, R<sup>384</sup>, R<sup>385</sup>, R<sup>386</sup>, R<sup>387</sup>, R<sup>388</sup>, R<sup>389</sup>, R<sup>390</sup>, R<sup>391</sup>, R<sup>392</sup>, R<sup>393</sup>, R<sup>394</sup>, R<sup>395</sup>, R<sup>396</sup>, R<sup>397</sup>, R<sup>398</sup>, R<sup>399</sup>, R<sup>400</sup>, R<sup>401</sup>, R<sup>402</sup>, R<sup>403</sup>, R<sup>404</sup>, R<sup>405</sup>, R<sup>406</sup>, R<sup>407</sup>, R<sup>40</sup>



### Formula III

prodrugs thereof, and the pharmaceutically acceptable salts of the compounds and the prodrugs, wherein:

B is N or C(Q<sup>1</sup>), where Q<sup>1</sup> is H or (C<sub>1</sub>-C<sub>2</sub>)alkyl;

L is *n*-propyl, phenyl, or  $\text{CH}_2$ -methylene,  $\text{CH}_2$ , wherein X is t-butyl, thienyl, thiazolyl or tetrahydrofuranyl, said  $\text{CH}_2$ -methylene- $\text{CH}_2$  or X being optionally mono-, di- or tri-substituted on aromatic carbon, independently with one to three chloro, fluoro, methoxy, difluoromethoxy, trifluoromethoxy, trifluoromethyl, or methyl;

R is carbonyl,  $(C_1-C_6)$ alkoxycarbonyl, tetrazolyl, 5-oxo-1,2,4-triazolyl, 5-oxo-1,2,4-oxadiazolyl,  $(C_1-C_6)$ alkylsulfonylcarbonyl or phenylsulfonylcarbonyl;

**R<sup>1</sup> is H, methyl, ethyl or propyl**

R<sup>2</sup> is H or (C<sub>2</sub> - C<sub>5</sub>) alkanoyl;  
R<sup>3</sup> is independently H, fluoro or methyl;

$R^4$  is  $H$ ,  $(C_1 - C_7)$  alkyl, or  $R^4$  and  $R^5$  are taken together to form a 5-9 membered carbocyclic ring, said alkyl being optionally monosubstituted and optionally mono-, di- or tri-substituted independently with one or three fluoro, chloro, methoxy, difluoromethoxy, trifluoromethoxy, trifluoromethyl or methyl;

carbonyl), (C<sub>2</sub>-C<sub>2</sub>)polyalkylacetonol, (C<sub>2</sub>-C<sub>2</sub>)polyalkylacetonol, G-acetol or G-acetonol, said (C<sub>1</sub>-C<sub>2</sub>)alkylsulfonol, (C<sub>2</sub>-C<sub>2</sub>)polyalkylsulfonol, (C<sub>2</sub>-C<sub>2</sub>)polyalkyl(C<sub>1</sub>-C<sub>2</sub>)alkylsulfonol, (C<sub>1</sub>-C<sub>2</sub>)alkyl(C<sub>2</sub>-C<sub>2</sub>)polyalkylacetonol, (C<sub>2</sub>-C<sub>2</sub>)polyalkyl(C<sub>1</sub>-C<sub>2</sub>)alkylacetonol optionally mono-, di- or tri-substituted on carbon independently with hydroxy, fluoro, chloro, methoxy, difluoromethoxy, trifluoromethoxy, trifluoromethyl or methyl;

Z is methylene, ethylene, propylene or ethenylene;

are *Ar*, *Ar*<sup>1</sup>, *Ar*<sup>2</sup>, *Ar*<sup>3</sup>, *Ar*<sup>4</sup>, *Ar*<sup>5</sup>, *Ar*<sup>6</sup>, *Ar*<sup>7</sup>, *Ar*<sup>8</sup>, *Ar*<sup>9</sup>, *Ar*<sup>10</sup>, *Ar*<sup>11</sup>, *Ar*<sup>12</sup>, *Ar*<sup>13</sup>, *Ar*<sup>14</sup>, *Ar*<sup>15</sup>, *Ar*<sup>16</sup>, *Ar*<sup>17</sup>, *Ar*<sup>18</sup>, *Ar*<sup>19</sup>, *Ar*<sup>20</sup>, *Ar*<sup>21</sup>, *Ar*<sup>22</sup>, *Ar*<sup>23</sup>, *Ar*<sup>24</sup>, *Ar*<sup>25</sup>, *Ar*<sup>26</sup>, *Ar*<sup>27</sup>, *Ar*<sup>28</sup>, *Ar*<sup>29</sup>, *Ar*<sup>30</sup>, *Ar*<sup>31</sup>, *Ar*<sup>32</sup>, *Ar*<sup>33</sup>, *Ar*<sup>34</sup>, *Ar*<sup>35</sup>, *Ar*<sup>36</sup>, *Ar*<sup>37</sup>, *Ar*<sup>38</sup>, *Ar*<sup>39</sup>, *Ar*<sup>40</sup>, *Ar*<sup>41</sup>, *Ar*<sup>42</sup>, *Ar*<sup>43</sup>, *Ar*<sup>44</sup>, *Ar*<sup>45</sup>, *Ar*<sup>46</sup>, *Ar*<sup>47</sup>, *Ar*<sup>48</sup>, *Ar*<sup>49</sup>, *Ar*<sup>50</sup>, *Ar*<sup>51</sup>, *Ar*<sup>52</sup>, 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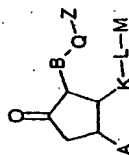
on sulfur, or Ar is a fully saturated ring having one or two oxo groups substituted independently from oxygen, sulfur and nitrogen; or Ar is a fully saturated ring having one or two heteroatoms selected from nitrogen, sulfur and oxygen, and partially or fully saturated five- or six-membered rings substituted independently, optionally having one to four heteroatoms selected independently from nitrogen, sulfur and oxygen, or a bicyclic ring consisting of three fused, independently partially saturated, fully saturated or fully unsaturated five- or six-membered rings, optionally having one to four heteroatoms selected independently from oxygen, sulfur and nitrogen, or a bicyclic ring consisting of two fused, independently partially saturated, fully saturated or fully unsaturated five- or six-membered rings, taken independently, optionally having one to four heteroatoms selected independently from nitrogen, sulfur and oxygen, or a bicyclic ring consisting of three fused, independently partially saturated, fully saturated or fully unsaturated five- or six-membered rings, optionally having one to four heteroatoms selected independently from nitrogen, sulfur and oxygen, and partially or fully saturated five- or six-membered rings substituted on carbon or one or two oxo groups substituted independently from oxygen, sulfur and nitrogen; or Ar is a fully saturated ring having one or two heteroatoms selected

Ar<sup>1</sup> and Ar<sup>2</sup> are each independently a partially saturated, fully saturated or fully unsaturated five- to eight-membered ring optionally having one to four heteroatoms selected independently from oxygen, sulfur and nitrogen, or a bicyclic ring consisting of two fused independently partially saturated, fully saturated or fully unsaturated five- or six-membered rings, taken independently, optionally, having one to four heteroatoms selected independently from nitrogen, sulfur and oxygen, or a bicyclic ring consisting of three fused independently partially saturated, fully saturated or fully unsaturated five- or six-membered rings, taken independently, optionally, having one to four heteroatoms selected independently from nitrogen, sulfur and oxygen, said partially or fully saturated ring optionally having one or two oxo groups substituted on carbon or one of two oxo groups substituted on sulfur.

[illegible]

V is a bond, thio(C<sub>1</sub>-C<sub>2</sub>alkylene), (C<sub>1</sub>-C<sub>2</sub>alkylenethio), (C<sub>1</sub>-C<sub>2</sub>alkylenoxy), oxy(C<sub>1</sub>-C<sub>2</sub>alkylene) or (C<sub>1</sub>-C<sub>2</sub>alkylene optionally mono- or di-substituted, when V is not a bond, independently with hydroxy or fluoro; and (iv) compounds of Formula IV

and said EP<sub>2</sub> receptor selective agonist is selected from compounds of Formula V:



### Formula IV

prodrugs thereof, and the pharmaceutically acceptable salts of the compounds and the prodrugs wherein:

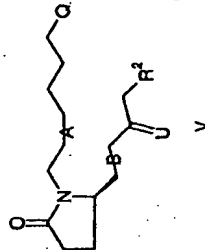
A is hydrogen or hydroxy;  
B is propylene, propenylene or propynylene;  
Q is propylene,  $\text{-CH}_2\text{OCH}_2\text{-}$ , thiazolo, pyridyl, phenyl or thianyl;  
Z is carbonyl, (C<sub>1</sub>-C<sub>3</sub>)alkoxy-carbonyl, tetrazolyl, 1,2,4-oxadiazolyl or 5-oxo-1,2,4-oxadiazolyl;  
K is ethylene or ethynylene;  
L is a bond or  $\text{-CO-}$ ;  
M is  $\text{-Ar'}$ ,  $\text{-Ar'1-VAr'2}$ ,  $\text{-Ar'1-SAr'2}$  or  $\text{-Ar'1-O-Ar'2}$  wherein Ar and Ar' are either

**Ar and Ar<sup>1</sup> are either**

(f) each independently a fully unsaturated five- to eight-membered ring optionally having one to four heteroatoms selected independently from oxygen, sulfur and nitrogen, or a bicyclic ring consisting of two fused partially saturated, fully saturated or fully unsaturated five- and/or six-membered rings, and/or a bicyclic ring consisting of two fused partially saturated, fully saturated or fully unsaturated five- and/or six-membered rings, taken independently, optionally bearing one to four substituents selected independently from nitrogen, sulfur and oxygen, any of said partially saturated heteroatoms substituted on carbon, or

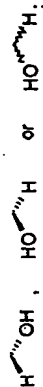
(g) each independently a fully unsaturated five- to eight-membered ring substituted on carbon, or

(h) each independently a fully saturated five- to eight-membered ring;

[illegible]

prodrugs thereof and pharmaceutically acceptable salts of said compounds or said prodrugs, wherein:

Q is COOR<sup>3</sup>, CONHR<sup>4</sup> or tetrazol-5-yl;  
A is a single or cis double bond;  
B is a single or trans double bond;  
U is



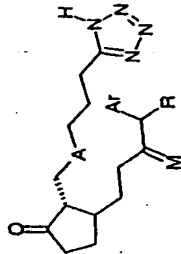
R<sup>2</sup> is  $\alpha$ -thienyl, phenyl, phenoxy, monosubstituted phenyl and monosubstituted phenoxy, said substituents being ethoxy, fluoro, phenyl, methoxy, trifluoromethyl or (C<sub>1</sub>-C<sub>3</sub>)alkyl;  
R<sup>3</sup> is hydrogen, (C<sub>1</sub>-C<sub>3</sub>)alkyl, phenyl or p-biphenyl;  
R<sup>4</sup> is COR<sup>5</sup> or SO<sub>2</sub>R<sup>5</sup>, and  
R<sup>5</sup> is phenyl or C<sub>1</sub>-C<sub>3</sub>alkyl.

3. The use as claimed in claims 1 to 2 wherein the condition is osteoporosis.

4. A pharmaceutical composition comprising an EP<sub>2</sub> receptor selective agonist as claimed in claims 1 and 2, an EP<sub>4</sub> receptor selective agonist as claimed in claims 1 and 2, and a pharmaceutically acceptable vehicle, carrier or diluent.

The use of an EP<sub>2</sub>/EP<sub>4</sub> receptor selective agonist, produg or pharmaceutically acceptable salt thereof in the preparation of a medicament for the treatment of a condition which presents with low bone mass in a mammal.

6. The use as claimed in claim 5 where the EP<sub>2</sub>/EP<sub>4</sub> receptor selective agonist is selected from compounds of the Formula VI:



VI

wherein A is ethylene or cis-vinylene; M is



R is hydrogen or methyl; and Ar is phenyl, wherein said phenyl is optionally monosubstituted with fluoro, chloro, bromo, trifluoromethyl, methyl, methoxy or phenyl.

7. The use as claimed in claims 5 to 6 where the condition is osteoporosis.

8. The kit comprising

- (a) a first dosage unit form comprising an EP<sub>2</sub> receptor selective agonist as claimed in claims 1 to 2 and a pharmaceutically acceptable vehicle, carrier or diluent.
- (b) a second dosage unit form comprising an EP<sub>4</sub> receptor selective agonist as claimed in claims 1 to 2 and a pharmaceutically acceptable vehicle, carrier or diluent.
- (c) a container where said dosage unit forms may be administered separately or together.



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(11) EP 1 121 939 A3

EUROPEAN PATENT APPLICATION

(12) (51) Int. Cl.: A61K 31/195, A61K 31/4015,  
A61K 31/41, A61K 45/06,  
A61P 19/08, A61P 19/10

(18) Date of publication A2:  
08.08.2001 Bulletin 2001/32

(21) Application number: 01300989.8

(22) Date of filing: 05.02.2001

<p>(84) Designated Contracting States: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE TR Designated Extension States: AL LT LV MK RO SI Priority: 07.02.2000 US 180635 P (71) Applicant: Pfizer Products Inc. Groton, Connecticut 06340 (US) (72) Inventors: • Cameron, Kimberly O'Keefe Groton, Connecticut 06340 (US)</p>	<p>• Thompson, David Duane Groton, Connecticut 06340 (US) • Lefker, Bruce Allen Groton, Connecticut 06340 (US) • Ko, Hua Zhu Groton, Connecticut 06340 (US) (74) Representative: Hayles, James Richard Pfizer Limited, Patents Department, Remgate Road Sandwich Kent CT13 9NJ (GB)</p>
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(54) Treatment of osteoporosis with EP<sub>2</sub>/EP<sub>4</sub> receptor selective agonists

(57) This invention is directed to methods and pharmaceutical compositions comprising prostaglandin agonists which are useful to prevent bone loss, restore or augment bone mass and to enhance bone healing including the treatment of conditions which present with low bone mass, such as osteoporosis, and/or bone defects in vertebrates, and particularly mammals, including humans. This invention specifically relates to methods and pharmaceutical compositions comprising combinations of EP<sub>2</sub> receptor selective agonist and EP<sub>4</sub> receptor selective agonists and to methods and pharmaceutical compositions comprising agonists which are agonists for both the EP<sub>2</sub> receptor and the EP<sub>4</sub> receptor.

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## PARTIAL EUROPEAN SEARCH REPORT

which under Rule 48 of the European Patent Convention EP 01 30 0999  
shall be considered, for the purposes of subsequent  
proceedings, as the European search reportEuropean Patent  
OfficeINCOMPLETE SEARCH  
SHEET CApplication Number  
EP 01 30 0999

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (INCL.7)
X	MEINREB H ET AL: "THE ANABOLIC EFFECT OF PGE2 IN RAT BONE MARROW CULTURES IS MEDIATED VIA THE EP4 RECEPTOR SUBTYPE" AMERICAN JOURNAL OF PHYSIOLOGY, AMERICAN PHYSIOLOGICAL SOCIETY, BETHESDA, MD, US, vol. 276, February 1999 (1999-02), pages 376-383, XP000952048 ISSN: 0882-9513 * page 377, column 1, paragraph 2 * * page 377, column 2, line 12 * * page 378, column 1, line 22 - page 378, column 2, line 2 * * page 379, column 2, line 10 - page 379, column 2, line 13 * * page 381, column 1, paragraph 2 * * figures 3, 7, 8 *	5,7	A61K31/195 A61K31/4015 A61K31/41 A61K45/06 A61P19/08 A61P19/18
Y	WU 98 27976 A (ROSATI ROBERT LOUIS : KE HUA ZHU (US); PRITZER (US); CAMERON KIMBERL) 2 July 1998 (1998-07-02) * the whole document *	1-4, 8 -/-	TECHNICAL FIELD SEARCHED (INCL.7) A61K A61P
<b>INCOMPLETE SEARCH</b> The Search Division considers that the present application, or one or more of its claims, should be examined, or may only be examined partially, for these reasons: Claims searched completely: Claims searched incompletely: Claims not searched: Reason for the limitation of the search: see sheet C			
Place of search MUNICH		Date of completion of the search 8 July 2003	Examiner Albrecht, S
DATE OF ENTRY OF OTHER DOCUMENTS X: particularly relevant foreign document Y: particularly relevant international document A: document of the same category C: non-written disclosure P: intermediate document T: theory or principle underlying the invention E: earlier patent document, but published on or after the filing date of the application D: document cited in the application L: document cited for other reasons S: member of the same patent family, corresponding document			

Claim(s) searched completely:

Claim(s) searched incompletely:  
1-8

Reason for the limitation of the search:

Present claims 1,3-5,7,8 relate to compounds defined by reference to a desirable characteristic or property, namely the capability of acting as an EP2 receptor selective agonist, an EP4 receptor selective agonist and as an EP2/EP4 receptor selective agonist. These claims cover all compounds having these characteristics or properties, and thus relate to an extremely large number of possible compounds, rendering a complete search impossible.

Furthermore, claims 2 and 6 relate to an extremely large number of possible compounds, defined by several Markush formulas. Support within the meaning of Article 84 EPC and/or disclosure within the meaning of Article 83 EPC is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Finally, the term "prodrug" used in claims 1,2,5 is vague and unclear and leaves in doubt as to the meaning of the technical feature (i.e. the compound) to which it refers. The lack of clarity is such as to render a meaningful complete search impossible.

Consequently, the search has been limited to the compounds exemplified in the documents W09827976 (compounds of formula I), W09919308 (compounds of formula II), EP0811321 (compounds of formula III), W09858911 (compounds of formula IV), EP1110949 (compounds of formula V) and US5932389 (compounds of formula VI), with due regard to the general concept of EP2 agonism, EP4 agonism and EP2/EP4 agonism.

The applicant's attention is drawn to the fact that some compounds may be already known to treat the diseases/disorders claimed by the applicant but are as yet not identified as EP2 receptor selective agonists. EP4 receptor selective agonists or as EP2/EP4 receptor selective agonists.





EP 1 121 939 A3

EP 1 121 939 A3

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EP 01 30 6999

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86-07-2063

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